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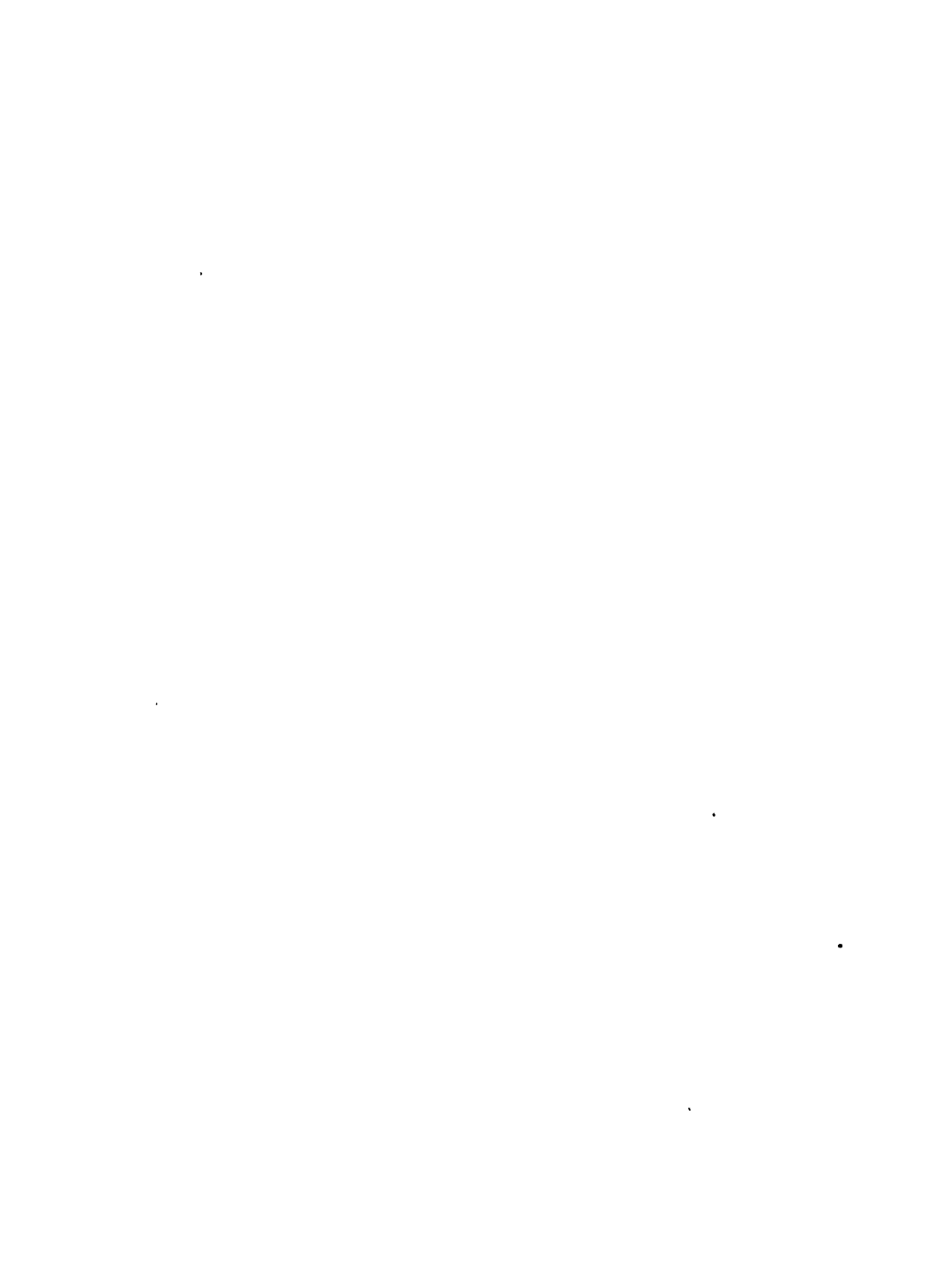
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# CONFORMATION AND ITS RELATION TO MILK PRODUCING CAPACITY IN JERSEY CATTLE<sup>1</sup>

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During the years in which observing men have kept cattle for milk production there has grown up the opinion that certain points in the conformation of the cow are directly associated with milk production. Perhaps no clearer statement in exemplification of this view can be made than that given by Mr. R. S. Curtis in his book on Cattle Judging (3):

When judging direct fitness for the block or for dairy purposes all breeding and ancestral records may be disregarded as all practical evidences of utility and quality are largely visible on the exterior of the animal.

Such opinions supported as they are by much of the literature of dairy cattle breeding and management are of especial interest to the biologist. The above quotation expresses as a conclusion one of the ultimate ends of biological investigation for many years, the resolving of the different life processes into terms of correlation between structure and function. The question arises, does this citation from Mr. Curtis express a truth, is it only measurably true or is it fallacious? Toward the solution of this question it is proposed to subject the scores for the conformation of a group of Jersey cows where the milk production of these cows is also known, to biometrical analysis in order to determine the amount of normal individual variation which exists and the relation this variation has to milk flow.

This study was undertaken because experience has shown that a reasonably accurate knowledge of the normal fluctuating variations of a character which is to be used as the basis of selection

<sup>1</sup> Paper from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 129.



is necessary if such selection is to accomplish the desired results. Previous studies (5, 11) have shown that these considerations have immediate bearing on studies involving milk flow as one of the component elements. Milk production is known to be a highly variable character controlled partly by the cow's own internal mechanism, and partly by environmental influences in its final expression. To arrive at any just conclusions regarding the merits of conformation it is necessary to have some knowledge of the influence of the variations of conformation and of milk flow in relation to each other. Such a point of view is entirely free of any preconceived theory as to what particular position one may hold as to the significance of the amount and kind of the variation.

#### MATERIAL AND METHODS

The present study is based on the material collected by the American Jersey Cattle Club in its Registry of Merit work compiled by Mr. R. M. Gow<sup>2</sup> (2). Part of this data has been published in volume I of the Registry of Merit. The larger portion has not been published. The published data gives the name and registry number, total score on a scale of 100, the milk production, butter-fat percentage, age, etc., of the animal. These records have been used by the author for a study of the relation of conformations of the cows at different ages, as measured by score, to the milk productions of the same cows.

The unpublished data so far as the author is aware have never been subject to analysis. These data are far superior to the published material in that the scores of the individual parts of the body are given. The score card used in this paper is the one officially adopted by the American Jersey Cattle club in 1903. The parts of the body considered together with the points allowed for a perfect score are reproduced below. The score actually given any part of the body of a cow represents the amount by which that part of the cow approaches the ideal dairy form as conceived of by the scorer.

<sup>2</sup> It is a pleasure to acknowledge the indebtedness of the author to Mr. R. M. Gow, Secretary of the American Jersey Cattle Club in furnishing a set of these score cards for this investigation. We are most grateful for the courtesy and coöperation shown by him and the officials under him.

**Points scored by cow:**

Name.....H. R. No.....

		COUNTS	
<b>Head, 7—</b>			
A—Medium size, lean; face dished; broad between eyes and narrow row between horns.....	}	4	.....
B—Eyes full and placid; horns small to medium, incurving; muzzle broad, with muscular lips; strong under jaw....	}	3	.....
<b>Neck, 5—</b>			
Thin, rather long, with clean throat; thin at withers.....		5	.....
<b>Body, 35—</b>			
A—Lung capacity, as indicated by depth and breadth through body, just back of fore legs .....	}	5	.....
B—Wedge shape, with deep, large paunch, legs proportionate to size and of fine quality.....	}	10	.....
C—Back straight to hip-bones .....		2	.....
D—Rump long to tail-setting and level from hip-bones to rump-bones.....	}	8	.....
E—Hip-bones high and wide apart; loins broad, strong .....		5	.....
F—Thighs flat and well cut out.....		3	.....
<b>Tail, 2—</b>			
Thin, long, with good switch, not coarse at setting-on.....		2	.....
<b>Udder, 28—</b>			
A—Large size and not fleshy.....		6	.....
B—Broad, level or spherical, not deeply cut between teats....		4	.....
C—Fore udder full and well rounded, running well forward of front teats.....	}	10	.....
D—Rear udder well rounded, and well out and up behind..		8	.....
<b>Teats, 8—</b>			
Of good and uniform length and size, regularly and squarely placed.....	}	8	.....
<b>Milk Veins, 4—</b>			
Large, tortuous and elastic.....		4	.....
<b>Size, 3—</b>			
Mature cows, 800 to 1,000 pounds.....		3	.....
<b>General Appearance, 10—</b>			
A symmetrical balancing of all the parts, and a proportion of parts to each other, depending on size of animal, with the general appearance of a high-class animal, with capacity for food and productiveness at pail.....		10	.....
		100	

A little thought will make it clear that these records differ from most scientific data in the following important aspect. Each part of the cow is compared with the ideal in the judge's mind. The number of points allowed, or the degree in which the cow before the judge measures up to his ideal, represents the cow's score. Two variables are thus introduced in the final recorded measure of the cow's conformation, the variation in the ideal type from judge to judge and the variation in the opinion of the judges as to how far a given defect is from the said ideal type. The case is something like that of classifying eye colors in human beings. We know that the ability of the different judges varies and that some may vary so far as to be entirely inaccurate but taking the large total number of observers into consideration the analysis of the data gives a good mean of how the average trained dairyman would classify this group of cattle with regard to conformation. The conclusions derived are therefore unique in giving the value of conformation as a guide to milk production as seen through the eyes of the average trained dairyman rather than those of the genius or of the mediocre man.

The data used for this study were complete for the following items.

- a. The judge scored all parts of the cow as indicated in the 1903 score card.
- b. All cows scored had Registry of Merit records for a period of 365 days.
- c. The cows' ages, milk production, butter-fat per cent and butter-fat were given.

Of the total number of records available 1674 records were found to be complete for the above data.

These records are exceptionally suited to the aims of this investigation. They are as near a random sample of the judgment of what constitutes the type of an ideal dairy cow as it would be likely to obtain. One hundred and forty different men have scored one or more of these animals. Most of these men are well known in the Jersey breed. The cows on which scores are taken are all registry of merit cows. The conclusions drawn from the data will consequently be representative of what constitutes the best of the Jersey breed.

The methods used are those in ordinary biometrical use. Sheppard's correction for the second moment has not been used in any of the calculations. Many of the frequencies are quite irregular due to mental habit of the observers centering observations on even numbers or on the fives. No effort has been made to smooth out these irregularities, each constant being calculated from the raw data as it stands.

To facilitate the analysis of the problem the issues may be restated in somewhat more concrete form.

a. How nearly does the representative cow of the Jersey Registry of Merit approach the ideal dairy form, as a whole and as to the various parts into which the body is divided?

b. What is the variation of these cows with respect to this average type?

c. What relation has this variation to the whole form (total score) to the milk production of the cow?

d. Does the variation of part of the body measure accurately or relatively accurately an increase or decrease in the milk production of that cow?

The data necessary to this analysis are found in the back of this paper. The physical constants of the distributions are given in table 1.

The means in table 1 bear directly on the first object of this investigation. In this group of 1674 Jersey Registry of Merit cows the average score was  $89.848 \pm 0.073$ , in other words the average cow of this group differed from the ideal Jersey cow by about 10 points. The amount by which the individual body parts of these average cows differ from the ideal Jersey cow is given in the fourth column. The greatest difference is found in the fore udder. This difference is no doubt due to the lack of size and symmetry of this part of the udder on many cows. The other parts of the average cow which differ most from the ideal form are the shape and size of the barrel and the general appearance (the symmetry and balance of the animal as a whole). The size of the average cow approached most nearly the ideal of any of the divisions used in the consideration of these animals.

TABLE 1  
Physical constants for the conformation and milk production of Jersey Registry of merit cows

CHARACTER	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	PERFECT CONFORMATION, —MEAN CONFORMATION	PERFECT CONFORMATION — MEAN CONFORMATION — × 100
<i>Head</i> —Medium size, lean, face dishd; broad between eyes and narrow between horns.	3.538±0.006	0.360±0.004	10.17±0.12	0.46	11.6
<i>Head</i> —Eyes full and placid; horns small to medium, incurving; muzzle broad, with muscular lips; strong under jaw.	2.638±0.008	0.458±0.005	17.36±0.20	0.36	12.1
<i>Neck</i> —Thin, rather long, with clean throat; thin at withers.	4.453±0.007	0.441±0.005	9.91±0.12	0.55	10.9
<i>Body</i> —Lung capacity, as indicated by depth and breadth through body, just back of fore legs.	4.644±0.006	0.348±0.004	7.50±0.08	0.36	7.1
<i>Body</i> — Wedge shape, with deep, large paunch, legs proportionate to size and of fine quality.	8.996±0.012	0.711±0.008	7.90±0.09	1.00	10.0
<i>Body</i> —Back straight to hip-bones.	1.764±0.004	0.222±0.003	12.60±0.15	0.24	11.8
<i>Body</i> —Rump long to tail-setting and level from hip-bones to rump-bones.	7.119±0.011	0.664±0.008	9.33±0.11	0.88	11.0
<i>Body</i> — Hip-bones high and wide apart; loins broad, strong.	4.596±0.006	0.380±0.004	8.27±0.09	0.40	8.1
<i>Body</i> —Thighs flat and well cut out.	2.732±0.005	0.272±0.003	9.94±0.12	0.27	8.9

<i>Tail</i> —Thin, long, with good switch, not coarse at setting-on.	1.826±0.003	0.200±0.002	10.96±0.13	0.17	8.7
<i>Udder</i> —Large size and not fleshy.	5.307±0.009	0.567±0.007	10.69±0.13	0.69	11.6
<i>Udder</i> —Broad, level or spherical, not deeply cut between teats.	3.447±0.007	0.429±0.005	12.43±0.14	0.55	13.8
<i>Udder</i> —Fore udder full and well rounded, running well forward of front teats.	8.452±0.017	1.011±0.012	11.97±0.14	1.55	15.5
<i>Udder</i> —Rear udder well rounded, and well out and up behind.	7.265±0.010	0.611±0.007	8.41±0.09	0.74	9.2
<i>Teats</i> —Of good and uniform length and size, regularly and squarely placed.	7.130±0.011	0.693±0.008	9.72±0.12	0.87	10.9
<i>Milk Veins</i> —Large, tortuous and elastic.	3.473±0.007	0.425±0.005	12.22±0.14	0.53	13.2
<i>Size</i> —Mature cows, 800 to 1,000 pounds.	2.868±0.003	0.163±0.002	5.68±0.07	0.13	4.4
<i>General appearance</i> —A symmetrical balancing of all the parts, and a proportion of parts to each other, depending on size of animal, with the general appearance of a high-class animal, with capacity for food and productiveness at pail.	9.004±0.013	0.761±0.009	8.45±0.09	1.00	10.0
Total score on conformation	89.848±0.073	4.443±0.052	4.95±0.06	10.15	10.2
Milk production . . . . .	7802.8±29.9	1812.2±21.2	23.22±0.28		

While the differences of the average Jersey cow from the ideal type are of much significance, they do not tell the whole story. The reason for this is that the points allowed for the different body divisions are not the same but are weighted differently. The question on which we desire information is the relative amount of deviation each given body part has as compared with what the ideal would be. This information may be approached and measured numerically in a number of ways. Perhaps the simplest and most easily understood formula for doing this is given below.

$$K = \frac{P.C. - M.C.}{P.C.} \times 100$$

Where  $K$  is a whole number ranging from 0 to 100. This constant measures the relative defect in the conformation of a given body part as compared with another in the average typed Jersey cow.  $P.C.$  equals the perfect conformation of the ideal cow and  $M.C.$  the mean conformation of the average cow.

Column five of table 1 presents this information for this group of Jersey cows. On the scale of this constant the average cow of the 1674 considered ranges from 4.4 to 15.5 units away from the ideal type in the different divisions into which the body conformation is classified.

The most seriously defective places or those which received most consideration from the judges pertained to the size of udder and its blood supply. Thus the fore udder was the furthest from the ideal type; the udder shape taken as a whole was the next part of the body most off type; the size and character of the milk veins were considered the next furthest off the ideal form. The parts into which conformation was divided which deviated least from the ideal were the size or weight of the cow. If the three main divisions into which the score card is divided are considered it is found that the body regions approach nearest to the ideal type; the head next and the udder is furthest removed from this ideal conformation. If the conformation as a whole is compared with the mammary development as a whole it is found that the body form approaches most nearly the ideal. From these con-

siderations it becomes plain that in the minds of these judges the part of the body requiring greatest development in this ideal milch cow is the mammary system as distinguished from the rest of the body.

The variation of this measure for conformation of a given body division is given by the standard deviation. The largest standard deviation for a given part of the body is that for the fore udder. A fairly close second to this is the variation of the general appearance. The part of the conformation exhibiting the lowest variation is the size of the body.

The characters which the judge compares with his ideal are essentially morphological characters. It is therefore of some interest and significance to consider the variability of these characters abstractly. This may be done by means of the coefficient of variation.

The lowest coefficient of variation is that for the total score, the coefficient of variation in this case being only  $4.95 \pm 0.06$ . The coefficients of variation for the individual parts of the conformation range from 5.68 to 17.36 with a mean of 10.19. The least variable part of the conformation was the size of the animal. The most variable was that part of the head included in the eyes, horns and muzzle. Considering the parts of the conformation having no direct relation to the organs of milk secretion the mean coefficient of variation was 9.84. The mean coefficient of variation was 10.91 for those parts of the conformation which dealt directly with the milk secreting function. Those parts of the body relating to the milk secreting function are consequently more variable than those relating to the rest of the body. This conclusion seems quite reasonable when it is remembered that the greater part of the mammary conformation relates to the development of the soft part of the body where as the greater number of the other parts making up the conformation relates to bone.

The variability of the milk production checks well with what has previously been found for a pure bred herd of the breed (6). When the variation of the milk production is compared with that of the parts into which the conformation is divided it is



TABLE 2

*Coefficients of variation for characters comparable with those here studied.*

CHARACTER	COEFFICIENT OF VARIATION	AUTHORITY
Area of comb (domestic fowl) . . . . .	39.97	Pearl and Pearl (12)
Weight of spleen (English males) . . . . .	38.21	Greenwood (8)
Milk production (Jersey Random sample herd) . . . . .	25.57	Gowen (6)
Milk production (Guernsey Advanced Registry) . . . . .	24.72	Gowen (6)
Milk production (Holstein-Friesian Advanced Registry) . . . . .	24.27	Gowen (5)
Milk production (Jersey Registry of Merit) . . . . .	23.22	
Heart weight (English males) . . . . .	22.22	Greenwood and Brown (9)
Weight of kidneys (English males) . . . . .	21.05	Greenwood and Brown (9)
Weight of liver (English males) . . . . .	20.82	Greenwood and Brown (9)
Body weight (English males) . . . . .	18.91	Greenwood and Brown (9)
Rev. maximum daily milk yield (for given age) . . . . .	17.998	Gavin (4)
Head—Eyes full and placid; horns small to medium, incurving; muzzle broad, with muscular lips; strong under jaw . . . . .	17.36	This paper
Weekly milk yield (Ayrshire cattle) . . . . .	17.08	Pearl and Miner (11)
Breathing capacity (English males) . . . . .	16.60	Pearson (13)
Body—Back straight to hip-bones . . . . .	12.60	This paper
Udder—Broad, level or spherical, not deeply cut between teats . . . . .	12.43	This paper
Milk Veins—Large, tortuous and elastic . . . . .	12.22	This paper
Udder — Fore udder full and well rounded, running well forward of front teats . . . . .	11.97	This paper
Tail—Thin, long, with good switch, not coarse at setting-on . . . . .	10.96	This paper
Udder—Large size and not fleshy . . . . .	10.69	This paper
Head—Medium size, lean; face dished; broad between eyes and narrow between horns . . . . .	10.17	This paper
Body—Thighs flat and well cut out . . . . .	9.94	This paper
Neck—Thin, rather long, with clean throat, thin at withers . . . . .	9.91	This paper
Teats—Of good and uniform length and size, regularly and squarely placed . . . . .	9.72	This paper
Body—Rump long to tail-setting and level from hip-bones to rump-bones . . . . .	9.33	This paper

TABLE 2—Continued

CHARACTER	COEFFICIENT OF VARIATION	AUTHORITY
General Appearance—A symmetrical balancing of all the parts, and a proportion of parts to each other, depending on size of animal, with the general appearance of a high-class animal, with capacity for food and productiveness at pail . . . . .	8.45	This paper
Udder—Rear udder well rounded, and well out and up behind . . . . .	8.41	This paper
Body—Hip-bones high and wide apart; loins broad, strong . . . . .	8.27	This paper
Brain weight (Bavarian males) . . . . .	8.12	Pearl (10)
Body—Wedge shape, with deep, large paunch, legs proportionate to size and of fine quality . . . . .	7.90	This paper
Body—Lung capacity, as indicated by depth and breadth through body, just back of fore legs . . . . .	7.50	This paper
Size—Mature cows, 800 to 1000 pounds . . . . .	5.68	This paper
Length of forearm (English males) . . . . .	5.24	Pearson and Lee (14)
Length of femur (French males) . . . . .	5.05	Pearson (13)
Total score on conformation . . . . .	4.95	This paper
Stature (English males) . . . . .	3.99	Pearson and Lee (14)

found that the milk yield variation is about twice as much as the variation of the judges estimate of the variability of the different parts making up conformation.

It is of significance to follow this comparison further. Table 2 gives the coefficients of variation of characters of a similar nature to those of conformation save that the coefficients in this material are measure or weight accurately in English or metric units.

Comparison of the coefficients of variation for milk production of the material used in this paper with that of a random sample Jersey herd and for the other cows in the advanced registries of the other breeds shows that so far as the variation of yield is concerned these data are entirely comparable with those of other dairy cattle in this and other breeds.

Some variation constants for bone growth and size data have been included in this table. It will be noted that the coefficients of variation for bone material are in general lower than are those for the scores on the conformation of the parts which require principally the difference in bone size from animal to animal in the make up of their variation constants. The smaller size of these coefficients of variation where actual bone measurements were used as compared with those judged against the ideal make it seem probable that the variability of the score for these parts is somewhat too large as compared with the actual variability as found on the animal.

Other data on variation included in this table pertains to the variation of the soft organs of the body in various species. These data are very variable ranging in value from 8.12 to 39.97. These constants cover such a range that the comparison of them with the data on the variation of the conformation of the soft part of the cow is of doubtful value. The data of this paper on the soft parts deal chiefly with the mammary system. The variation constants for them seem to fall well within the range of the same kinds of data on other species although in general they are grouped near the lower end of this range.

#### THE CORRELATION OR INTERRELATION OF THE CONFORMATION OF THE BODY PARTS WITH THE VARIATION OF THE MILK SECRETION

This phase of the subject is perhaps the most interesting since it measures and gives the first reliable means of distinguishing between the value of the different points on conformation as they are indicative of the cow's milking capacity. Table 3 gives this information in terms of correlation. The arrangement of the material is according to the amount of correlation which exists between milk production and the conformation of the given part.

The arrangement of this table shows clearly the items covered under conformation which have value in determining what the future milk production of the cow will be. Only one part of the

TABLE 3

*Correlation between conformation and milk yield*

CHARACTER CORRELATED	CORRELATION COEFFICIENT	CORRELATION COEFFICIENT P. E. F.
Milk yield and total score.....	0.1941±0.0160	12.13
Milk yield and milk veins—Large, tortuous and elastic.....	0.1908±0.0160	11.93
Milk yield and udder—Large size and not fleshy.....	0.1906±0.0160	11.91
Milk yield and udder—Rear udder well rounded, and well out and up behind.....	0.1710±0.0161	10.62
Milk yield and body-wedge shape, with deep, large paunch, legs proportionate to size and of fine quality.....	0.1657±0.0161	10.29
Milk yield and general appearance—Sym- metrical balancing of all the parts, and a proportion of parts to each other, depend- ing on size of animal, with the general appearance of a high-class animal, with capacity for food and productiveness at pail.....	0.1147±0.0164	6.99
Milk yield and body-thighs flat and well cut out.....	0.0885±0.0164	5.40
Milk yield and body—Rump long to tail- setting and level from hip-bones to rump- bones.....	0.0862±0.0165	5.22
Milk yield and udder—Fore udder full and well rounded running well forward of front teats.....	0.0777±0.0165	4.71
Milk yield and teats—Of good and uniform length and size, regularly and squarely placed.....	0.0671±0.0165	4.07
Milk yield and head—Medium size, lean; face dished; broad between eyes and narrow be- tween horns.....	0.0671±0.0165	4.07
Milk yield and tail—Thin, long, with good switch, not coarse at setting-on.....	0.0634±0.0165	3.84
Milk yield and udder—Broad, level or spheri- cal, not deeply cut between teats.....	0.0615±0.0165	3.73
Milk yield and size—Mature cows, 800 to 1,000 pounds.....	0.0611±0.0165	3.70
Milk yield and body—Hip-bones high and wide apart; loins broad, strong.....	0.0589±0.0165	3.57
Milk yield and neck—Thin, rather long, with clean throat; thin at withers.....	0.0499±0.0165	3.02

TABLE 3—Continued

CHARACTER CORRELATED	CORRELATION COEFFICIENT	CORRELATION COEFFICIENT P. E. F
Milk yield and head—Eyes full and placid; horns small to medium, incurving; muzzle broad, with muscular lips; strong under jaw.....	0.0419±0.0165	2.54
Milk yield and body—Lung capacity, as indi- cated by depth and breadth through body, just back of fore legs.....	0.0222±0.0166	1.34
Milk yield and body—Back straight to hip- bones.....	-0.0697±0.0165	4.22

conformation has a minus value when correlated with the milk production of the cows. Put in words this means that as judged by these men the cow which had the *backs straight to hip bones* were very slightly poorer milk producers than those which were not so straight.

All correlation coefficients but two were more than three times the probable error of said coefficient. There are then 15 parts contributing toward the ideal conformation which are indicative of the high milk producers. The total score most nearly represented the milk producing capacities of the cow.

Of the separate divisions into which conformation is divided the *milk veins—large tortuous, and elastic* distinguish the high producer from the low producer most accurately. This conclusion finds interesting conformation in the work of Aldrich and Dana (1). This work dealt with nearly 600 cows. Among the items considered in relation to milk flow were (a) size of wells, (b) diameter of milk veins, (c) length of milk veins.

The correlation coefficients on these data where accurate measurements in English units were used, were (a) Aldrich  $0.151 \pm 0.031$ , Dana  $0.262 \pm 0.027$ ; (b) Aldrich  $0.193 \pm 0.036$ , Dana  $0.282 \pm 0.027$ ; (c) Aldrich  $0.003 \pm 0.066$ , Dana  $0.211 \pm 0.042$ . These correlations where actual measurements are used are quite close to the results obtained from the data of this paper. The conclusion seems justified therefore that the condition of the milk vein is to some extent an indicator of milk yield.

It may be well to mention an experiment performed by Graves of the Oregon Experiment Station (7), which might to some be considered a contradiction to this conclusion. In this experiment the Station veterinarian ligated the milk veins on an Ayrshire and on a Holstein-Friesian cow. No appreciable diminution of milk flow occurred in either of these cows after the operation was performed and the shock of the operation was over. Graves points out that there are two sets of veins carrying the blood from the udder, the so-called anterior and posterior mammary veins. In ligation only the anterior set are tied off. He says,

This experiment proves that the posterior mammary veins are capable of carrying all the blood away from the udder. It suggests to us that some cows may have the posterior vessels exceptionally well developed and the exterior mammary or milk veins poorly developed, and yet as much blood may be carried to the udder with the materials for the manufacture of milk as in the cow with the network of milk veins on her body.

It seems entirely likely that this experiment explains why the correlation coefficients between the milk veins and milk production is relatively low. The experiment does not prove that the anterior milk veins are not a fairly good indicator of the cow's milking capacity for it is entirely probable that while under stress the posterior veins can take care of all the blood going to the udder still under normal conditions it is equally probable that the size of these veins is proportional to the amount of blood that is necessary for the veins to carry. The relatively elastic nature of veins and the ease of their regeneration lend further strength to this view. The conclusion previously drawn from the data of this paper are, consequently, sound in showing that the condition of the milk veins is an indicator of the milking possibilities of the cow.

Close seconds to the condition of the milk veins as an indicator of the cow's possible milk yield are the size and character of the udder and the shape of the rear udder. The body size and shape together with the general appearance of the cow are next most indicative of her milk yield. It is interesting to note that the

size of the udder plays a much more important part in the yield than does the shape and contour of the udder. Likewise it seems that the appearance of the udder is more important than is the general form of the body taken as a whole. These facts substantiate the view that a cow with a large capacious barrel and large, elastic udder is likely to be a good milker.

These correlation coefficients are on the whole quite low. In this connection it is of interest to compare the relative value of correlation coefficients with those for actual milk yields over short periods. The problem may be stated thus.

a. What is the relation between the milk production of a short period (say seven days) in a lactation and that for the whole lactation?

b. What is the relation between the milk production of a short period and the milk production of a subsequent whole lactation of which the short period is not a part?

In answering this question the author quotes from some unpublished data soon to be put in bulletin form. Figure 1 shows the results of this comparison.

This figure shows clearly the much superior merit of a milk record even though it be for only seven days as compared with conformation in distinguishing the superior from the inferior dairy cow. The correlation coefficients are arranged in order of their value. This arrangement so far as the items of conformation are concerned is the same as that of table 3. The range in values of the correlation coefficients for milk yield of the seven day test with the milk yield of the year test in another lactation is  $+0.8470$  to  $0.3351$ . For the seven day test with the year test of the same lactation the range is  $+0.8360$  to  $-0.1157$ . For the parts considered in determining the conformation the range is  $+0.1941$  to  $-0.0697$ .

The average correlation coefficient for the seven day test of Holstein-Friesian cattle and the 365 day test of which the seven day test is a part is  $+0.570$ . The average correlation coefficient for the seven day test and 365 day test of which the seven day test is not a part is  $+0.550$ . In other words a seven day test is a much better measure of a cow's ability at the pail than is

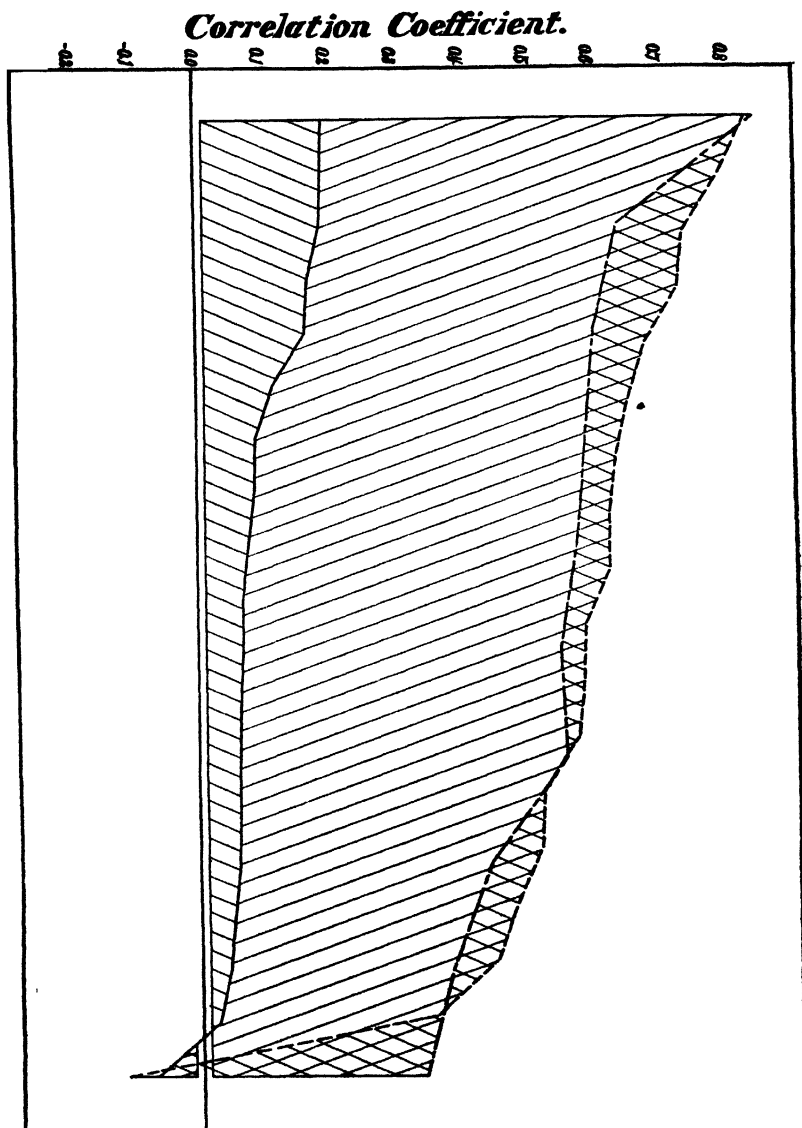


FIG. 1. COMPARATIVE VALUES OF THE CORRELATION COEFFICIENTS

Between (a) conformation and year milk production, (b) seven day test and year milk production where the seven day milk production is a component of the year milk production; and (c) seven day test and year milk production in which the seven day milk yield is in another lactation than the year lactation. The lines designating these relations are (a) ———, (b) ————, (c) ————.



TABLE 4  
*Constants for the linearity of regression of tables 5 to 23 of appendix*

	$r$	$\eta$	$\eta^2 - r^2$	$\frac{\eta^2 - r^2}{P_0 \eta^2 - r^2}$
Milk yield and head—Medium size, lean; face dishd; broad between eyes and narrow between horns.	0.0671±0.0165	0.1268±0.0163	0.0116±0.0035	3.31
Milk yield and head—Eyes full and placid; horns small to medium, incurving; muzzle broad, with muscular lips, strong under jaw.	0.0419±0.0165	0.1048±0.0163	0.0092±0.0031	2.97
Milk yield and neck—Thin, rather long, with clean throat; thin at withers.	0.0499±0.0165	0.1120±0.0163	0.0101±0.0032	3.16
Milk yield and body—Lung capacity, as indicated by depth and breadth through body, just back of fore legs.	0.0222±0.0166	0.0678±0.0164	0.0041±0.0022	1.86
Milk yield and body—Wedge shape, with deep, large paunch, legs proportionate to size and of fine quality.	0.1657±0.0161	0.1959±0.0160	0.0109±0.0035	3.11
Milk yield and body—Back straight to hip-bones.	-0.0697±0.0165	0.0926±0.0162	0.0037±0.0020	1.85
Milk yield and body—Rump long to tail-setting and level from hip-bones to rump-bones.	0.0862±0.0165	0.1091±0.0162	0.0045±0.0022	2.05
Milk yield and body—Hip-bones high and wide apart; loins broad, strong.	0.0589±0.0165	0.0733±0.0163	0.0019±0.0015	1.27
Milk yield and body—Thighs flat and well cut out.	0.0885±0.0164	0.1173±0.0162	0.0059±0.0026	2.27
Milk yield and tail—Thin, long, with good switch, not coarse at setting-on.	0.0634±0.0165	0.1128±0.0162	0.0087±0.0031	2.81
Milk yield and udder—Large size and not fleshy.	0.1906±0.0160	0.2281±0.0156	0.0157±0.0041	3.83
Milk yield and udder—Broad, level or spherical, not deeply cut between teats.	0.0615±0.0165	0.0983±0.0162	0.0050±0.0026	2.27

Milk yield and udder—Fore udder full and well rounded, running well forward of front teats.	0.0777±0.0165	0.1316±0.0162	0.0113±0.0035	3.23
Milk yield and udder—Rear udder well rounded, and well out and up behind.	0.1710±0.0161	0.2272±0.0156	0.0224±0.0049	4.57
Milk yield and teats—Of good and uniform length and size, regularly and squarely placed.	0.0671±0.0165	0.1264±0.0163	0.0115±0.0035	3.29
Milk yield and milk veins—Large, tortuous and elastic.	0.1908±0.0160	0.2089±0.0156	0.0072±0.0028	2.57
Milk yield and size—Mature cows, 800 to 1,000 pounds.	0.0611±0.0165	0.0910±0.0162	0.0045±0.0022	2.05
Milk yield and general appearance—A symmetrical balancing of all the parts, and a proportion of parts to each other, depending on size of animal, with the general appearance of a high-class animal, with capacity for food and productiveness at pail.	0.1147±0.0164	0.1534±0.0161	0.0104±0.0034	3.06
Total score.....	0.1941±0.0160	0.2198±0.0156	0.0106±0.0034	3.12

the total score or any part of the cow's conformation as judged by the average trained dairyman. This general relation between the milk yields of the various length of periods has been found to hold for the other breeds (6).

Before gathering together the conclusions it may be well to present the evidence to show that the regressions on which these correlation coefficients are based are all linear. The linearity of this regression is determined by the relation of the correlation coefficient and the correlation ratio. This relation is given in table 4.

Table 4 shows the correlation ratio for the conformation of each part of the body when correlated with milk yield to be approximately equal to the correlation coefficient for the same variables. The difference between the correlation ratio squared and the correlation coefficient squared is slight. The highest of these differences is only  $0.0224 \pm 0.0049$ . This difference deals with the regressions of the conformation of the rear udder on milk yield. When the differences of the correlation ratio squared minus the correlation coefficient squared are compared with their probable errors as is done in the last column of this table it is found that out of the nineteen differences only nine are more than three times their probable errors. Besides this the highest difference is only 4.57 times its probable error. It is doubtful indeed if nineteen correlation tables could be selected at random from material known to be linear in its regression and have the result conform more nearly than the tables of this paper.

The results of this analysis on which the conclusions are based are consequently free from any bias due to the type of regression lines not being linear.

#### SUMMARY

This paper presents a biometrical analysis of the relation of conformation to the milk producing capacity of the Jersey cow. Exceptional data have been made available to this Station for the solution of this problem by the courtesy of Mr. R. M. Gow. These data give the exact scores of 1674 registry of merit Jersey cows as determined by about 140 judges.

The mean conformation as measured by score is given for the cow as a whole and for the parts into which it is divided. Within this group of Jersey cows the average score was  $89.848 \pm 0.073$ . The average Jersey cow was therefore about 10 points below the ideal Jersey cow. When this measure of the conformation as a whole is analyzed in terms of its parts, it was found that the fore udder differed most from the ideal type.

When considered in abstract terms it was found that the most seriously defective parts of the body in the minds of the judges had to deal with the mammary system, its size and blood supply. Of those parts which dealt with the body proper the least ideal was the barrel.

The variation of the different body parts is compared by means of the standard deviation and the coefficient of variation. The most variable part of the body included the eyes, horns, and muzzle, the least variable the size of the body.

The variability is further compared with characters of similar nature to those of conformation save that the variability was determined on data measured or weighed accurately in English or metric units. Bone material was in general found to vary less than the scores assigned to parts of the body depending chiefly on variations in bone length. The amount of this difference was slight, however. The variation of the udder parts was found to be at the lower end of the range of variation of other soft parts of the body.

Correlation coefficients for milk yield with the conformation as a whole and for the various parts were determined. The correlation coefficients ranged from  $-0.0697 \pm 0.0165$  to  $0.1941 \pm 0.0160$ . Out of the nineteen correlations only one was minus in value; seventeen were more than three times their probable error. The total score had the highest correlation with milk yield. The parts of the conformation having a distinctly significant relation to milk production of the cow were the milk veins, size and condition of udder, the size and shape of rear udder, the shape and size of barrel and the general appearance of the cow.

The relative merits of conformation as a guide to the milk producing capacity of a cow and a short time milk record are

considered. The results show that a seven day test has a correlation coefficient with the year milk yield of the cow of approximately  $2\frac{1}{2}$  times that of the conformation or any part of the conformation. The short test consequently is superior to the conformation as a guide to milk production.

Constants for the regression of each correlation table are calculated and the regression shown to be linear.

The raw data are given in appendix tables 5 to 23.

TABLE 5

*Correlation surface for variables milk yield (365 days) and the conformation of the cow taken as a whole*

MILK	WHOLE CONFORMATION															TOTAL
	98-100	96	94	92	90	88	86	84	82	80	78	76	74	72	70-72	
<i>pounds</i>																
17,900-16,900					1											1
16,900				1												1
15,900																
14,900			6	2	2		2		1							13
13,900			2	1	2	1	1									7
12,900	1	1	5	6	3		2		1							19
11,900	1	4	7	15	13	5		2		1						48
10,900		9	23	15	19	12	5	4	2		1					90
9,900	5	9	27	35	38	35	22	8	4	9	4					196
8,900	3	16	45	57	48	39	25	21	10	7	6	1	1	1		280
7,900	1	13	45	63	82	73	48	37	20	14	4	7	2			409
6,900		8	25	60	76	78	44	30	22	22	6	3		1	1	376
5,900	1	3	21	30	39	37	37	13	6	12	4		1		1	205
4,900		3		9	6	2	2	1	4	1						28
3,900-2,900								1								1
Total.....	12	66	206	294	329	282	188	117	70	66	25	11	4	2	2	1674

TABLE 6

Correlation surface for variables milk yield (365 days) and the conformation of head, medium size, lean; face dishd; broad between eyes and narrow between horns

MILK	HEAD											TOTAL				
	3 8-4.0	3.6	3.4	3.2	3.0	2.8	2.6	2.4	2.2	2.0	1.8		1.6	1.4	1.2	1.0
<i>pounds</i>																
17,900-16,900	1															1
16,900			1													1
15,900																
14,900	4	4	3		1	1										13
13,900	5	1	1													7
12,900	1	6	9	3												19
11,900	23	4	15	3	3											48
10,900	34	18	19	6	3	8		2								90
9,900	63	33	43	20	5	25	2	2	1		1				1	196
8,900	98	50	81	10	5	31	1	2		2						280
7,900	113	72	118	25	14	60	2	5			3					409
6,900	90	70	129	19	11	50	2	2								376
5,900	46	51	74	12	4	15		2	1							205
4,900	7	3	10	1		6					1					28
3,900-2,900					1											1
Total.....	485	312	503	99	47	156	7	15	2		7				1	1674

TABLE 7

Correlation surface for variables milk yield (365 days) and the conformation of head, eyes full and placid; horns small to medium, incurving; muzzle broad, with muscular lips; strong under jaw

MILK	HEAD										TOTAL	
	2 3-3.0	2 6	2 4	2 2	2 0	1 8	1 6	1 4	1 2	1 0		0 8-1 0
<i>pounds</i>												
17,900-16,900	1											1
16,900		1										1
15,900												
14,900	6	3	2	1		1						13
13,900	2	2	3									7
12,900	8	10	1									19
11,900	22	8	13		1	4						48
10,900	34	34	16	2		3		1				90
9,900	88	42	40	13		11		1				196
8,900	116	73	58	15		12	3	3				280
7,900	143	101	99	20	1	42	1				2	409
6,900	119	95	99	31		31	1					376
5,900	78	46	54	15	2	8		2				205
4,900	11	7	8	1		1						28
3,900-2,900	1											1
Total.....	629	422	393	98	4	113	5	7			3	1674

TABLE 8

*Correlation surface for variables milk yield (365 days) and the conformation of neck, thin, rather long, with clean throat; thin at withers*

MILK	NECK											TOTAL
	4.5-5.0	4.0	4.4	4.2	4.0	3.8	3.6	3.4	3.2	3.0	2.8	
<i>pounds</i>												
17,900-16,900	1											1
16,900	1											1
15,900												
14,900	6	2	4			1						13
13,900	3	2	1	1								7
12,900	5	3	5	4		1		1				19
11,900	14	7	10	3		11					3	48
10,900	23	18	23	6		15	1	2			2	90
9,900	59	25	55	12	3	32	4	4			1	196
8,900	83	45	75	23	7	33	1	9			2	280
7,900	102	70	116	20	5	66	12	10	1		6	409
6,900	81	60	104	25	9	64	6	19	1		6	376
5,900	49	29	78	13		26	2	6			1	205
4,900	6	1	13	2	1	4	1					28
3,900-2,900						1						1
Total.....	433	262	484	109	25	254	27	51	2		21	1674

TABLE 9

*Correlation surface for variables milk yield (365 days) and the conformation of body, lung capacity, as indicated by depth and breadth through body, just back of fore legs*

MILK	BODY											TOTAL
	4.5-5.0	4.0	4.4	4.2	4.0	3.8	3.6	3.4	3.2	3.0	2.8	
<i>pounds</i>												
17,900-16,900	1											1
16,900			1									1
15,900												
14,900	5	5	3									13
13,900	5	1	1									7
12,900	7	6	4									19
11,900	28	6	12		2							48
10,900	47	13	16	3		7		2	1			90
9,900	102	20	40	12	3	17		2	2		1	196
8,900	133	34	66	14	1	24	4	3			1	280
7,900	217	37	89	24	1	35	4	1			1	409
6,900	172	52	84	20	2	36	4	2			3	376
5,900	111	27	39	15	2	7	2	1			1	205
4,900	13	2	11			2						28
3,900-2,900	1											1
Total.....	842	203	366	88	11	129	16	11			7	1674

TABLE 10

*Correlation surface for variables milk yield (365 days) and the conformation of body, wedge shape, with deep, large pauch, legs proportionate to size and of fine quality*

MILK	BODY													TOTAL
	9.7-10.0	9.4	9.1	8.8	8.5	8.2	7.9	7.6	7.3	7.0	6.7	6.4	6.1	
<i>pounds</i>														
17,900-16,900	1													
16,900				1										
15,900														
14,900	5	2	1	4				1						
13,900	1	4		1			1							
12,900	4	3	5	5		1	1							
11,900	22	5	3	12		4	2							
10,900	25	23	4	20	1	7	8							
9,900	54	28	9	53	4	16	24	1	2		4	1		
8,900	76	50	8	66	5	32	32	3	3		4	1		
7,900	94	45	18	117	12	54	51	5	8	1	3			
6,900	74	53	21	95	11	57	48		11		6			
5,900	25	19	5	78	7	29	36	1	1		4			
4,900	4	4	1	6		7	4			1	1			
3,900-2,900				1										
Total. . . .	385	236	75	459	40	207	207	11	27	2	22	2	1	1674

TABLE 11

*Correlation surface for variables milk yield (365 days) and the conformation of body, back straight to hip-bones*

MILK	BODY										TOTAL
	18-2.0	1.6	1.4	1.2	1.0	0.8	0.6	0.4	0.2	0.0-2	
<i>pounds</i>											
17,900-16,900	1										1
16,900		1									1
15,900											
14,900	8	3	1			1					13
13,900	5	1	1								7
12,900	9	4	5	1							19
11,900	28	10	8	1		1					48
10,900	48	23	17	1				1			90
9,900	112	38	36	2	1	7					196
8,900	166	49	40	9		15				1	280
7,900	261	75	59	2		12					409
6,900	242	75	50	1	2	6					376
5,900	128	43	28	1	1	4					205
4,900	24	3	1								28
3,900-2,900			1								1
Total.....	1032	325	247	18	4	46		1		1	1674



TABLE 12

Correlation surface for variables milk yield (365 days) and the conformation of body, rump long to tail-setting and level from hip-bones to rump-bones

MILK	BODY													TOTAL	
	7.7-8.0	7.4	7.1	6.8	6.5	6.2	5.9	5.6	5.3	5.0	4.7	4.4	4.1		3.8-4.1
<i>pounds</i>															
17,900-16,900				1											1
16,900					1										1
15,900															
14,900	4	4	4				1								13
13,900	1	3	1	2											7
12,900	3	8	3	1	1		3								19
11,900	9	19	6	8	2	2	2								48
10,900	22	25	11	17	5	6	3				1				90
9,900	49	40	26	30	11	18	17		2		2			1	196
8,900	61	69	30	54	7	25	30	1	2			1			280
7,900	97	87	43	81	14	34	41	1	1	1	9				409
6,900	61	80	54	64	17	42	45		6		7				376
5,900	38	48	28	47	8	13	15		4	1	3				205
4,900	7	6	2	6	2	2	3								28
3,900-2,900				1											1
Total.....	352	389	208	312	68	142	160	2	15	2	22	1		1	1674

TABLE 13

Correlation surface for variables milk yield (365 days) and the conformation of body, hip-bones high and wide apart; loins broad, strong

MILK	BODY																TOTAL
	4.8-5.0	4.6	4.4	4.2	4.0	3.8	3.6	3.4	3.2	3.0	2.8	2.6	2.4	2.2	2.0	1.8-2.0	
<i>pounds</i>																	
17,900-16,900						1											1
16,900		1															1
15,900																	
14,900	1	5	5			1	1										13
13,900	5	1	1														7
12,900	8	5	5		1												19
11,900	31	5	11	1													48
10,900	40	12	27	3	1	7											90
9,900	87	28	43	6	4	25		1	1		1						196
8,900	138	33	61	17	1	22	2	4			1					1	280
7,900	165	49	92	25	3	61	2	7			5						409
6,900	178	45	81	19	4	42	4	2			1						376
5,900	82	26	59	7	3	23	1	1			2						205
4,900	8	9	3	1		7											28
3,900-2,900			1														1
Total.....	743	219	389	79	17	189	11	15	1		10					1	1674

TABLE 14

Correlation surface for variables milk yield (365 days) and the conformation of body, thighs flat and well cut out

MILK	BODY										TOTAL	
	2.8-3.0	2.6	2.4	2.2	2.0	1.8	1.6	1.4	1.2	1.0		0.8-1.0
<i>pounds</i>												
17,900-16,900	1											1
16,900	1											1
15,900												
14,900	11	2										13
13,900	5	1	1									7
12,900	15	1		3								19
11,900	35	6	4	1		2						48
10,900	59	16	8	2		5						90
9,900	124	33	25	6		8						196
8,900	182	42	36	10		8	1				1	280
7,900	224	76	67	13	3	24	1				1	409
6,900	211	74	55	13	3	14	2	3			1	376
5,900	109	43	31	12		10						205
4,900	17	5	3	2		1						28
3,900-2,900		1										1
Total.....	994	300	230	62	6	72	4	3			3	1674

TABLE 15

Correlation surface for variables milk yield (365 days) and the conformation of tail, thin, long, with good switch, not coarse at setting-on

MILK	TAIL										TOTAL
	1.9-2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.0	
<i>pounds</i>											
17,900-16,900	1										1
16,900		1									1
15,900											
14,900	10		2		1						13
13,900	7										7
12,900	8	3	2		3	3					19
11,900	30	10	4		1	2					48
10,900	64	5	8			11					90
9,900	121	15	30	1	3	17		3			196
8,900	180	22	42	6	5	16	2	2			280
7,900	255	22	74	4	6	41	1	1			409
6,900	202	30	74	8	9	38		4		11	376
5,900	109	13	44	4	8	20		4		3	205
4,900	15	6	5			2					28
3,900-2,900	1										1
Total.....	1003	127	285	23	36	150	3	14		33	1674

TABLE 16

Correlation surface for variables milk yield (365 days) and the conformation of udder, large size and not fleshy

MILK	UDDER															TOTAL	
	5.8-6.0	5.6	5.4	5.2	5.0	4.8	4.6	4.4	4.2	4.0	3.8	3.6	3.4	3.2	3.0		2.8-3.0
<i>pounds</i>																	
17,900-16,900																	1
16,900		1															1
15,900																	
14,900	4	1	2	2	1	1	1				1						13
13,900	2	1	2	2			2										7
12,900	10	2	1	1		2	2				1						19
11,900	22	4	7	3		8	2		1		1						48
10,900	40	10	11	10	5	7	2	2	2		1						90
9,900	73	17	30	20	6	28	7	8			5		2				196
8,900	103	39	37	23	4	44	7	10	2	1	6		3			1	280
7,900	117	40	59	28	15	83	26	18	2	1	15	1	2			2	409
6,900	73	26	67	34	18	77	27	24	5	4	21						376
5,900	33	12	33	21	9	48	16	18	2	4	9						205
4,900	4	3	4	3	1	7	1	3			2						28
3,900-2,900						1											1
Total.....	481	156	253	147	59	306	91	84	14	10	62	1	7			3	1674

TABLE 17

Correlation surface for variables milk yield (365 days) and the conformation of udder, b road, level or spherical, not deeply cut between teats

MILK	UDDER															TOTAL
	3.8-4.0	3.6	3.4	3.2	3.0	2.8	2.6	2.4	2.2	2.0	1.8	1.6	1.4	1.2	1.0	
<i>pounds</i>																
17,900-16,900						1			.							.
16,900		1														
15,900																
14,900	4	2	3	1		2					1					
13,900	1	1	3			1	1									
12,900	5	5	5	1		3										
11,900	17	8	8	3	1	10		1								
10,900	32	10	26	7	3	9		2	1							
9,900	62	25	55	9	6	30	6				2					1
8,900	79	42	74	16	7	39	8	7	5		3					
7,900	112	45	109	27	25	75	7	5			4					
6,900	81	52	109	20	15	69	7	6			8					
5,900	53	22	58	15	11	35	2	6	2		1					
4,900	7	4	6	2	2	6	1									
3,900-2,900					1											
Total.....	453	217	456	110	71	280	32	27	8		19					1

TABLE 18

Correlation surface for variables milk yield (365 days) and the conformation of udder, fore udder full and well rounded, running well forward of front teats

MILK	UDDER															TOTAL
	9.5-10.0	9.0	8.5	8.0	7.5	7.0	6.5	6.0	5.5	5.0	4.5	4.0	3.5	3.0	2.5-3.0	
<i>pounds</i>																
17,900-16,900			1												1	
16,900	1														1	
15,900																
14,900	3	4	2		3		1								13	
13,900	2	1	2		1		1								7	
12,900	1	8	5	2	1		2								19	
11,900	12	8	15	2	6	1	3		1						48	
10,900	18	17	19	16	14	2	4								90	
9,900	29	31	47	22	47	9	5	3	3						196	
8,900	48	43	83	23	44	12	15	3	7	1	1				280	
7,900	62	57	102	52	68	17	31	7	4		7	2			409	
6,900	54	43	105	51	69	20	19	5	5		3		1		376	
5,900	33	23	48	41	40	4	8	3	4				1		205	
4,900	3	1	13	4	5		1				1				28	
3,900-2,900		1													1	
Total . . . . .	266	237	442	213	298	65	90	21	24	1	12	2	2	1	1674	

TABLE 19

Correlation surface for variables milk yield (365 days) and the conformation of udder, rear udder well rounded, and well out and up behind

MILK	UDDER													TOTAL
	7.7-8.0	7.4	7.1	6.8	6.5	6.2	5.9	5.6	5.3	5.0	4.7	4.4	4.1	
<i>pounds</i>														
17,900-16,900	1													1
16,900	1													1
15,900														
14,900	7			4	1						1			13
13,900	3	2			2									7
12,900	11	1	2	1	2	2								19
11,900	18	15	3	9		2	1							48
10,900	49	18	7	8	4	3	1							90
9,900	77	34	25	34	3	11	10				2			196
8,900	111	59	33	41	4	19	11		2					280
7,900	131	80	43	84	9	30	25	1	3		1			409
6,900	94	58	55	84	15	37	27	1	3		2			376
5,900	42	42	20	50	10	26	11				2	1		205
4,900	8	6	4	4	2	2	2							28
3,900-2,900				1										1
Total.....	553	315	192	320	52	132	88	2	8		8	1	3	1674

TABLE 20

Correlation surface for variables milk yield (365 days) and the conformation of teats, of good and uniform length and size, regularly and squarely placed

MILK	TEATS											TOTAL	
	7.5-8.0	7.0	6.5	6.0	5.5	5.0	4.5	4.0	3.5	3.0	2.5		2.0-2.5
<i>pounds</i>													
17,900-16,900					1								1
16,900			1										1
15,900													
14,900	5	2	4				2						13
13,900	2	2	2						1				7
12,900	11	4	4										19
11,900	21	12	7	4	4								48
10,900	44	23	18	1	3		1						90
9,900	85	51	37	11	8	3	1						196
8,900	133	64	54	13	10	3	1		2				280
7,900	146	91	102	32	31	1	3		3				409
6,900	127	98	87	29	28	3	1		2		1		376
5,900	68	62	38	19	15	1	2						205
4,900	7	11	7	1	2								28
3,900-2,900		1											1
Total.....	649	421	361	110	102	11	11		8		1		1674

TABLE 21

Correlation surface for variables milk yield (365 days) and the conformation of milk veins, large, tortuous and elastic

MILK  pounds	MILK VEINS										TOTAL	
	3.8-4.0	3.6	3.4	3.2	3.0	2.8	2.6	2.4	2.2	2.0		1.8-2.0
17,900-16,900						1						1
16,900		1										1
15,900												
14,900	7	2	1	3								13
13,900	1		3	2		1						7
12,900	10	3	4									19
11,900	24	5	9	4	2	2	1					48
10,900	41	13	23	2	1	9	1				1	90
9,900	80	22	47	13	7	26		1				196
8,900	104	29	66	18	20	32	5	1	1		4	280
7,900	148	33	97	28	24	63	3	10	1		2	409
6,900	85	31	79	53	30	67	13	6	5		7	376
5,900	52	17	52	21	17	28	11	2	1		4	205
4,900	5		12	3	4	3		1				28
3,900-2,900							1					1
Total.....	557	156	393	147	107	232	35	21	8		18	1674

TABLE 22

*Correlation surface for variables milk yield (365 days) and the conformation of size, mature cows, 800 to 1,000 pounds*

MILK	SIZE														TOTAL
	2.8-3.0	2.6	2.4	2.2	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6	0.4	0.2	
<i>pounds</i>															
17,900-16,900	1														1
16,900	1														1
15,900															
14,900	13														13
13,900	7														7
12,900	19														19
11,900	46	1	1												48
10,900	84	3	1			2									90
9,900	191	3	2												196
8,900	264	7	4	2		2									280
7,900	379	13	6	4		6			1						409
6,900	349	11	8	1	1	3	2				1				376
5,900	185	7	5	5		3									205
4,900	25		2	1											28
3,900-2,900	1														1
Total.....	1565	45	29	13	1	16	2		1		1			1	1674

TABLE 23

*Correlation surface for variables milk yield (365 days) and the conformation of general appearance,—a symmetrical balancing of all the parts, and a proportion of parts to each other, depending on size of animal, with the general appearance of a high class animal, with capacity for food and productiveness at pair*

MILK	GENERAL APPEARANCE																TOTAL
	9.7-10.0	9.4	9.1	8.8	8.5	8.2	7.9	7.6	7.3	7.0	6.7	6.4	6.1	5.8	5.5	5.2	
<i>pounds</i>																	
17,900-16,900	1																1
16,900				1													1
15,900																	
14,900	5	6	1	1													13
13,900	1	1		4			1										7
12,900	3	8		2		3	2				1						19
11,900	21	11	3	8			4				1						48
10,900	26	14	6	32		6	2		2		1	1					90
9,900	55	31	6	55	5	16	18		6		3					1	196
8,900	75	44	9	63	10	34	28	4	3		4	1		3		2	280
7,900	93	58	20	109	14	36	61	2	6		6	2		2			409
6,900	67	51	15	121	10	47	49	1	9		5					1	376
5,900	41	35	6	61	3	29	20	1	2	1	6						205
4,900	7	4	1	3	1	8	3		1								28
3,900-2,900											1						1
Total.....	395	263	67	460	43	179	188	8	29	1	28	4		5		4	1674

## REFERENCES

- (1) ALDRICH, A. M., AND DANA, J. W. 1917 The relation of the milk veins system to production. In *Bul. 202, Vermont Agricultural Experiment Station*, p. 1-24, fig. 3.
- (2) American Jersey Cattle Club. 1911 Register of Merit of Jersey Cattle, vol. i to iv.
- (3) CURTIS, ROBERT S. 1915 The fundamentals of live stock judging and selection. New York, p. 450, fig. 180.
- (4) GAVIN, W. F. 1913 The interpretation of milk records. In *Jour. Roy. Agric. Soc.*, lxxiii, p. 1-22.
- (5) GOWEN, JOHN W. 1919 Variations and mode of secretion of milk solids. In *Jour. of Agri. Research*, xvi, no. 3, p. 79-102.
- (6) GOWEN, JOHN W. Studies in milk secretion. V. On the variations and correlations of milk secretion with age. To appear in *Genetics*.  
GOWEN, JOHN W. VI. On the variations and correlations of butter-fat percentage with age in Jersey cattle. To appear in *Genetics*.
- (7) GRAVES, R. R. 1916 An experiment with milk veins. In *Hoard's Dairyman*, iii, no. 2, p. 687 and 717, fig. 6.
- (8) GREENWOOD, M. 1904 A first study of the weight, variability, and correlation of the human viscera, with special reference to the healthy and diseased heart. In *Biometrika*, iii, p. 63-84.
- (9) GREENWOOD, M., AND BROWN, T. W. 1913 A second study of the weight, variability and correlation of human viscera. In *Biometrika*, ix, p. 473-485.
- (10) PEARL, RAYMOND 1905 Biometrical studies on man. I. Variations and correlations in brain weight. In *Biometrika*, iv, p. 13-104.
- (11) PEARL, RAYMOND, AND MINER, JOHN R. 1919 The variation of the milk of Ayrshire cows in quantity and fat content of their milk. In *Ann. Rept. Maine Agricultural Experiment Station*, 1919, p. 57-64.
- (12) PEARL, RAYMOND, AND PEARL, MAUD DEWITT 1909 Data on variation in the comb of the domestic fowl. In *Biometrika*, vi, p. 420-432.
- (13) PEARSON, KARL 1899 Mathematical contributions to the theory of evolution. V. On the reconstruction of the stature of prehistoric races. In *Phil. Trans. R. Soc. London*, vol. 192A, p. 162-244, 2 pl.
- (14) PEARSON, KARL AND LEE, ALICE. 1897 Mathematical contributions to the theory of evolution. On the relative variation and correlation in civilized and uncivilized races. In *Proc. R. Soc. London*, vol. lxi, no. 375, p. 343-357.

# EXPERIMENTS WITH AND PRACTICAL APPLICATION OF HEAT STERILIZATION FOR ALL PARTS OF MILKING MACHINES

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## INTRODUCTION

The last twenty-five years have seen marked improvements in the construction of milking machines. Today a number of makes have been sufficiently perfected and are milking cows so successfully that they may be considered a practical success from the mechanical standpoint.

Labor difficulties due to the war gave a great impetus to the installation of milking machines. Today the capital invested in the manufacturing plants as well as in machines distributed on dairy farms is so large that investigation into the difficulties encountered in their operation is greatly needed.

A review of the literature of this country and abroad on such investigations shows it to be quite extensive. A great many experiments have been initiated to ascertain the mechanical difficulties and the relative production that can be secured with their use as compared with hand milking. With these factors we were not concerned but have devoted our attention to ascertaining the best method of eliminating excessive numbers of bacteria in machine drawn milk.

The sanitary side of milking machine operation was investigated early in their development, at which time not nearly so much was known about the origin of the bacterial flora in milk as at present. Consequently it must be recognized that some of the early bacteriological work on this subject is no longer of any value in the light of our present day knowledge.



PUBLISHED INVESTIGATIONS ON BACTERIA IN MACHINE DRAWN MILK AND THE USE OF VARIOUS STERILIZING AGENTS

Among the most important works on this subject the following will be mentioned. F. C. Harrison (1) of the Guelph Experiment Station in Canada reported, in 1897, a rather extensive series of bacteriological experiments on milk drawn by hand and by the Thistle machine. He concluded that machine drawn milk contained more bacteria than hand drawn milk under the same conditions. This he attributed to contamination of teat cups with hairs and dirt from the cow, inability of the rubber parts of the machine to stand sterilization by steaming or scalding, and letting the cups fall to the floor in changing from cow to cow.

In 1902, Bordas and Raczkowski (2), in France, demonstrated a higher bacterial content in machine drawn milk even when special antiseptic precautions were taken. Their experiments, however, include only seven bacterial counts in all and therefore are not conclusive.

In 1907, W. A. Stocking (3), reported experiments with various methods of cleaning milking machines. Satisfactory results were obtained by means of heat but even boiling in water for three quarters of an hour did not keep the rubber parts sterile. The bacterial count of the milk produced after this treatment was well below 10,000 per cubic centimeter. Placing the rubber parts, after washing, in brine for several hours reduced the bacterial count of machine drawn milk to about half of that drawn by hand. In this case he scalded the rubber parts just before use after taking them out of the brine solution or just before putting them into the solution. Heat, therefore, was probably the main factor in getting the machines sufficiently clean to produce all of his low count samples.

At the Kansas Station, in 1906, Erf (4) in a general treatise on the construction and evolution of the milking machine reports several tests with antiseptic solutions for sterilizing parts. Formaldehyde, boric acid and lime were tried with the result that while formaldehyde was the most efficient it was not practical

and lime was concluded to be the most satisfactory. After soaking in these solutions the rubber parts were thoroughly rinsed in boiling water before use.

In 1907, Stocking and Mason (5) reported work on this problem in which steam, Gold Dust solution 1-300, lime water, borax 1 pound in 15 quarts of water, 2.5 to 3.5 per cent formalin and 10 per cent brine were used. They were only able to keep the tubes in a satisfactorily sterile condition with steam and formaldehyde. The steam was applied without pressure for one hour and quickly injured the rubber tubing so it could not be used.

Hastings and Hoffman (6), at the Wisconsin Agricultural Experiment Station, in 1906, showed that with lime solution as a disinfectant for rubber parts 150 samples of machine drawn milk gave an average lower count than 137 hand drawn samples. This was done on the station herd where better care was probably taken of the machines than would have occurred under ordinary farm conditions. The lime solution was of a strength equal to a twentieth normal solution of alkali. Care was used to obtain perfectly fresh unslacked lime and to maintain an excess in the vessel.

Elizabeth Meek (7) reported a series of counts with hand drawn and machine drawn milk. At first the machines were merely being rinsed out successively with cold, tepid and hot water when the milk drawn by them was high in bacterial content. Later the machines were steamed and the hose and cups stored in brine solution after being thoroughly washed with a tube brush. After this treatment the bacterial content of the milk produced by them was reduced to approximately that found in the hand milked product.

Brainard (8) in a general bulletin on clean and sanitary milk reports bacterial counts of machine drawn milk. For several days after the machine was installed at the Virginia Experiment Station the count ranged from 32,000 to 35,000. It was then not used for three days and when again placed in operation the count increased to 1,250,000. He then prepared a lime water bath for the rubber parts and in three days the count was down

and thereafter ranged between 34,000 and 100,000, which compared favorably with the hand drawn milk.

Harding, Wilson and Smith (9), in 1909, concluded that the most important item in obtaining quality of product was the immersing of the rubber parts in 10 per cent brine or similar solution between milkings. This, they state, is "many times more efficacious than the most careful steaming." This conclusion has not been borne out under actual field conditions in this state.

Wing (10), in 1913, showed that many colonies would develop on plates made from 15 per cent brine in which rubber tubing from milking machines had been kept. A number of substances were then added to the brine including hydrogen peroxide, alcohol, permanganate of potash, formaldehyde, vinegar, acetic acid, copper sulphate and, finally, chloride of lime. She concluded that brine would not keep parts sterile but that when chloride of lime was added this result was practically obtained. It was necessary, however, to frequently add fresh chloride of lime. With sterile machine parts she was able to produce low count milk.

A recent and extensive experimental work on milking machines as a source of bacteria in milk has been published by Reuhle, Breed and Smith (11). They have carried on elaborate experiments in maintaining milking machines in a bacteria free condition by using antiseptic solutions, some of which are 10-13 per cent brine solution, chloride of lime, the two together, lime water, cold running water and a proprietary germicide, "Montanin." Satisfactory results are claimed for all of these but the conclusion is made that chloride of lime in saturated brine is the most practical and efficient. The work is noticeably lacking in experiments on the value of heat sterilization for rubber parts which is surprising in the light of the finding of Haecker and Little (12), in 1908; Stocking (3), in 1907; Edwards (13), in 1907, and the widespread unsatisfactory results from the bacteriological standpoint with all the chemical preparations when used under ordinary dairy ranch conditions.

## LIMITATIONS OF CHEMICAL SOLUTIONS AS STERILIZING AGENTS

An efficient antiseptic bath for milking machine rubber parts must meet certain fundamental requirements. First, it must keep the rubber parts bacteriologically clean; second, it must not deteriorate the rubber rapidly; third, leave no deposit in the teat cups and tubes; fourth, leave no objectionable taste, odor or preservative in the milk; fifth, be fairly stable.

All of the preparations except the hypochlorites have proved unsatisfactory and have been discarded for the latter in this section of the country. Hypochlorites in the form of chloride of lime, Javelle's or Labarague's solution (U. S. P.) and the well known proprietary preparations such as B. K., Chlorax, etc., are now recommended by the milking machine companies and generally used in California.

The two factors which constitute the vast majority of causes for discarding milking machines at the present time are: First, the increase in udder troubles, and second, the high bacterial count in machine drawn milk. The latter is of great importance where dairies are selling their product for market milk supply under a bacterial count grading system or where a premium is paid for sweet cream delivered to butter making plants.

Milking machine companies are themselves to blame for many machines being discarded because of the common practice of their selling agents to state that the machines need not regularly be taken apart for cleaning and that dipping in chlorine solution is all that is necessary.

Agents selling the proprietary chlorine preparations have been even more extravagant in their claims of the value of their solutions for sterilizing milking machine parts.

Ordinary chloride of lime (calcium hypochlorite-bleaching powder) contains about 25 to 35 per cent available chlorine which is the highest free chlorine content of any of these preparations. Javelle's or Labarague's solution, United States Pharmacopeia, and the well known proprietary chlorine preparations contain from about 2.5 to 5 per cent available chlorine. They are clear solutions, however, and therefore much more easily mixed up ready for use than chloride of lime.

The power of chlorine solutions to act as a disinfectant depends first, on the available chlorine content and, second, on the chemical and physical properties of the material to be disinfected. Granting that the chlorine content is present, many observations in the dairy industry, under various conditions, have definitely shown that these solutions are not so efficient in the presence of a considerable accumulation of milk residue as when this has been properly removed by thorough washing.

For example, it has been demonstrated that chlorine solution run into the last rinse water tank in a milk bottle washing machine, in a high dilution, was remarkably efficient in reducing the bacterial content of the water and the finished bottles. The same or greater strength solution showed little efficiency when run over milk cooling apparatus, pipe lines and containers which were noticeably greasy when the hand was passed over them. This is probably due to its lack of penetrating power. The presence of organic matter such as milk residue rapidly deteriorates chlorine solution by using up the chlorine in oxidation. After all the chlorine is utilized, the disinfectant properties of such a solution are nil and bacteria may even multiply in it in great numbers.

To show the reduction in chlorine by oxidation in the presence of milk or rubber the following tests were made with the assistance of C. H. McCharles, Chemist of the State Food and Drug Laboratory. Three  $1\frac{1}{2}$  gallon glass jars were each partially filled with one gallon of water to which chlorine solution was added in the proportion of 3 ounces to 5 gallons. This is about the proportion usually recommended for milking machine parts. The chlorine solution before dilution showed 2.35 per cent available chlorine, and when added to the water in the above proportion, the fluid in the jars contained about one part of available chlorine to each 10,000 parts of water.

Jar 1 was not disturbed; jar 2 had 2 cc. of milk added daily; jar 3 had some rubber tubing placed in it.

Titration for chlorine content were made each day, the results of which are shown in table 1.

Bacterial counts of the solution in jar 2 showed no growth on June 12 and 13. On June 19, ten days after mixing up the solution, it showed a bacterial content of 210,000 per cubic centimeter. Two cubic centimeters of milk had been added each day from June 9 to 17 inclusive.

A second test was conducted using only two jars. The solution was mixed in the same proportions. Jar 1 was left undis-

TABLE 1

	JAR 1	JAR 2	JAR 3
June 9, 1919..... {	2.80 cc. 0.0099 gm.	0.90 cc. 0.0032 gm.	3.00 cc. 0.0106 gm.
June 10, 1919..... {	2.80 cc. 0.0099 gm.	0.45 cc. 0.0016 gm.	1.30 cc. 0.0046 gm.
June 11, 1919..... {	2.80 cc. 0.0099 gm.	0.30 cc. 0.0011 gm.	0.90 cc. 0.0032 gm.
June 12, 1919..... {	2.70 cc. 0.0096 gm.	0.20 cc. 0.0007 gm.	0.65 cc. 0.0023 gm.
June 13, 1919..... {	2.70 cc. 0.0096 gm.	0.20 cc. 0.0007 gm.	0.30 cc. 0.0010 gm.
June 14, 1919..... {	2.70 cc. 0.0096 gm.	0.15 cc. 0.0005 gm.	0.15 cc. 0.0005 gm.
June 16, 1919..... {	2.60 cc. 0.0092 gm.	0.10 cc. 0.0003 gm.	0.05 cc. 0.0001 gm.

cc. = cubic centimeters N/10 sodium hyposulphite equivalent to 100 cc. sample.

gm. = grams of available chlorine per 100 cc. of sample. The fourth decimal of the gram measurement represents parts of chlorine per million of solution.

turbed as a check and in jar 2 the rubber tubing was placed and also 2 cc. of milk daily. The results of this test are given in table 2.

A bacterial count of the solution in jar 2, made June 27, 1919, showed 600 bacteria per cubic centimeter.

These tests show that both milk and rubber reduce the chlorine content of solutions containing it. The more milk residue that

adheres to the tubing, the quicker the chlorine becomes utilized. Many crocks used for the solution on dairy ranches are of such a size that the tubing cannot lay out straight in them. It is the usual practice for the parts to be forced down under the solution so that there are bends in the tubing, in which case, air instead of solution may remain in contact with the lumen of the tubes. After use for some time the rubber tubing shows checking or slight cracks on its surfaces which can be enlarged and readily seen by distending the tubing. These crevices, although

TABLE 2

	JAN 1	JAN 2
June 19, 1919.....{	3.45 cc. 0.0122 gm.	3.10 cc. 0.011 gm.
June 20, 1919.....{	3.40 cc. 0.0121 gm.	1.55 cc. 0.0055 gm.
June 21, 1919.....{	3.40 cc. 0.0121 gm.	0.60 cc. 0.0021 gm.
June 23, 1919.....{	3.40 cc. 0.0121 gm.	0.20 cc. 0.0007 gm.
June 24, 1919.....{	3.40 cc. 0.0121 gm.	0.15 cc. 0.0005 gm.
June 25, 1919.....{	3.30 cc. 0.018 gm.	0.075 cc. 0.0002 gm.

hardly visible, become filled with milk during the milking and furnish innumerable points for bacterial multiplication out of reach of the chlorine solution which, as stated above, seems to have little or no power of penetration through a layer of milk residue.

As examples of the failure of this means of sterilizing rubber parts the following might be mentioned:

In 1913, a dairy using milking machines attempted to get dairy certification under the San Francisco Medical Milk Commission and failed to meet the bacterial requirements on account of depending on chemical sterilization of parts.

On October 1, 1916, the California state milk grading law went into effect. This law allows a maximum of 200,000 bacteria per cubic centimeter for grade "A" milk to be pasteurized and 1,000,000 bacteria per cubic centimeter for grade "B" milk to be pasteurized. This law was at once enforced in Los Angeles and dairies operating milking machines could not meet the bacterial requirements by using chemical solutions. The machines had to be discarded, the milk degraded to butter fat or the parts, including rubber tubing and inflations, sterilized by heat.

#### EXPERIMENTS TO SHOW THE PRACTICABILITY AND EFFICIENCY OF HEAT STERILIZATION

The early work of Stocking (3) with heat sterilization by placing the tubing on a steam jet, scalding just prior to use and boiling for three-quarters of an hour was found to be most efficient but not recommended on account of the destruction of the parts.

Edwards (13), in 1907, found boiling and proper cleaning efficient in keeping bacteria down. Haecker and Little (11) found lime water inefficient unless accompanied by boiling and painstaking cleanliness of parts. They stated that rubber tubing at the station dairy, subjected to daily boiling, remained in good condition for more than a year.

In spite of these definite conclusions that heat was essential, it has not been put into general practical use on account of the belief that the rubber would be injured. With the quality of rubber now in use it is possible to boil it daily for a considerable time without destroying its usefulness.

To test this, a piece of rubber tubing as supplied by one of the milking machine companies was boiled for twenty to thirty minutes each week day from December 13, 1917, to May 28, 1918, without injuring it to such a degree that it would not be usable. The inflations, however, are more susceptible to the destructive influences of heat than the tubing.

Realizing the great efficiency of pasteurization at 145°F. in destroying 95 to 99 per cent of the bacteria ordinarily present in milk, it was thought that some temperature between 145° F. and



212°F. could be found at which a pasteurization of milking machine rubber goods would render them practically sterile and yet prolong the usefulness of the rubber for a longer period than when subjected to boiling.

Experiments with heat sterilization were begun on dairy "A." This is a large dairy milking about 200 cows and supplying a "guaranteed" milk to the San Francisco Bay cities. The bacterial limit of this grade of milk is 25,000 per cubic centimeter. The Sharples milking machines were in use. The original plan of sterilization of the parts on this dairy was to put the tubes and teat cups in a low pressure steam sterilizer, but depreciation of the rubber was rapid. A galvanized iron tank, 4 feet long, 1 foot wide and  $1\frac{1}{2}$  feet deep, without any lid, was then installed and the parts placed in water in the tank and the temperature raised by steam to between 160° and 180°F. for fifteen to twenty minutes. A series of bacterial counts were made on the parts so sterilized and on the milk drawn by the machines.

In this work the tests of the sterility of the parts were conducted by running sterile water through the apparatus to be tested. The amount of water used in each test was 100 cc. Plates were made according to the standard methods of the American Public Health Association from the rinsings.

In the first series of counts on the sterility of the apparatus the plates made from the teat cups and connected rubber tubing showed colonies too numerous to accurately count in the undiluted rinse water. The pails were also unsterile but the rinse water from them did not contain as many bacteria as that from the rubber ware. One milk pail and one stripping pail showed 197 and 653 bacteria per cubic centimeter of rinse water, respectively, which were the lowest counts obtained. A second series of similar tests were made November 12, 1917, except that the rinse water was diluted 1-100 before plating. Twelve samples of water run through the teat cups and connected rubber tubing showed bacterial counts ranging from 205 to 24,200 per cubic centimeter. At the conclusion of this test a lid was ordered for the sterilizer tank and a definite temperature of 180°F. for fifteen to thirty minutes was maintained and followed as a routine pro-

cedure. On December 13, 1917, a third series of counts were made. Five sterile rinse water samples were run through the connected apparatus. The counts on these rinse water samples, using 1 cc. undiluted in making the plates, ranged from 6 to 60 bacteria per cubic centimeter.

The bacterial counts of the milk drawn through this apparatus on the different dates are given in table 3.

One sample of bottled milk was taken on November 5 which showed 11,500 bacteria per cubic centimeter.

TABLE 3

SAMPLE FROM	COUNT 1st UNIT	COUNT 2d UNIT	COUNT 3d UNIT	STRIppINGS	DATE
1st pail milk	24,300	5,700	10,300	26,200 1st string	November 5, 1917
2d pail milk	7,000	12,600	9,500	23,100 1st string	November 5, 1917
3d pail milk	5,400	8,800	12,800	7,600 2d string	November 5, 1917
4th pail milk		7,400	5,700	70,900 3d string	November 5, 1917
5th pail milk	7,200	9,000		26,000 3rd string	November 5, 1917
1st pail milk	7,300	2,450	7,700	9,000 1st string	November 12, 1917
2nd pail milk	2,450	1,800	1,750	17,500 3rd string	November 12, 1917
3d pail milk	2,900	4,250	500	16,000 2d string	November 12, 1917
1st pail milk		7,500	900	No strippings	December 13, 1917
2d pail milk	3,500	4,750	1,850	taken	December 13, 1917
3d pail milk	3,000				December 13, 1917

The milk counts on November 5 and 12 show that the sterilization, even though very incomplete, was sufficient to produce a milk well under 25,000 bacteria per cubic centimeter, the limit for the grade.

An examination of table 3 will show little change in the bacterial count of the milk on December 13, even though the tubes and teat cups at this time were practically sterile. This is accounted for by the dilution of the bacteria on the previous occasions with the large volume of milk and the fact that the actual number of bacteria in the rubber tubes and teat cups, even though not countable when 1 cc. of undiluted rinse water was plated as in the first series of tests, was relatively small.

In the spring of 1918 scarcity of labor made it necessary for dairy "B" to put in milking machines. This is a certified dairy milking 225 cows, the product of which is sold in San Francisco and the maximum count of this grade is 10,000 per cubic centimeter. The step was taken with considerable apprehension for fear the quality of the product would be reduced.

From the experience gained with dairy "A," this dairy was advised to and did install a galvanized iron tank similar to that described on dairy "A" for sterilizing the rubber parts of the machines.

On this dairy both the Empire and Perfection milking machines were in operation from the beginning. The method of cleaning the machines was as follows. Each unit was attached to the suction line and a vacuum established in the pail. The teat cups were then alternately raised and lowered into a pail of water with the pulsator running. Three to four gallons of water were run through the machines in this manner to clean out the milk. The rubber parts were then disconnected from the pails and the teat cups and short rubber tubes taken off the claw to facilitate brushing. At first, the inflations were taken out of the teat cup shells but this was soon stopped on account of the difficulty of reassembling. All parts were placed in warm water containing soda ash cleaning powder and scrubbed both ways with long brushes for this purpose. They were then rinsed in clear water to remove the soda ash and reassembled ready for use. A plug was inserted in the air line at its junction with the claw and the whole placed in the sterilizer. Water was run in until they were submerged and the pulsator lids were placed on top of the rubber tubing in such a way as to hold it down in the water and at the same time prevent the pulsator from getting flooded. Those machines that have the pulsator slightly elevated or off the lid entirely have an advantage in that there is no danger of getting water in the delicate mechanism. Occasionally a lid will warp in this process of sterilizing, after which it will no longer make a tight fit on the pail and a vacuum cannot be secured.

The cover was placed on the sterilizer and the steam turned on till the temperature reached 190°F., where it was maintained

for fifteen to thirty minutes. After sterilization was completed, the water was allowed to cool and the contents left undisturbed in the tank until the next milking time.

Further investigation is necessary to definitely determine whether it is better to remove the tubes or leave them in the water. Some evidence is present to show that keeping them moist prolongs their period of usefulness over allowing them to dry quickly after sterilizing. From the bacteriological standpoint no difficulty has been experienced with undestroyed bacteria multiplying after an optimum temperature is reached.

In May and June, 1918, a series of bacterial counts were made at dairy "B" as shown in table 4. On this dairy each machine unit milks 45 cows.

The tinware on this ranch is regularly sterilized in an autoclave under pressure. In all, 113 samples of machine milked milk were collected and counted. Two samples were over 10,000; the highest being 18,000.

During the year from June 1, 1918, to June 1, 1919, the highest count that was obtained in official samples for dairy "B" was 15,500 and an average of all samples taken was 6000. The rubber parts on this dairy have remained in use from two to four months—the tubes being longer lived than the inflations.

The following experimental work was conducted on dairy "C." This dairy was of the type of the ordinary market milk dairy, operated by a Portuguese dairyman and the milk shipped to be pasteurized in San Francisco. Perfection type of milking machines were in use. The general practice of cleaning the machines was to rinse out the milk with cold water by letting the machines run with the teat cups immersed in a pail of water. The long tubes and teat cups were brushed in warm water containing a cleaning powder but the teat cups were not disconnected from the claw. The air line was plugged and the whole coiled in a large earthenware jar containing B. K. solution between milkings. No careful sterilization of the tinware was done, although a boiler and steam jet were present for the purpose.

A bacterial count was made daily with few exceptions from November 1, 1918, to January 20, 1919. The sample was al-

TABLE 4

	SAMPLE OF					
	1st unit	2d unit	3d unit	4th unit	5th unit	Hand strip
<i>May 21, 1918</i>						
Rinsings of teat cups.....	1	0	1	0	0	
1st pail milk.....	3,200	2,100	18,000	1,150	6,400	5,700
2d pail milk.....	4,500	3,150	3,150	2,250	1,000	1,650
3d pail milk.....	2,950	4,700	2,250	5,100	2,550	7,650
<i>May 23, 1918</i>						
Rinsings of teat cups.....	3	2	4	2	5	
1st pail milk.....	2,300	2,300	9,550	2,450	450	17,500
2d pail milk.....	12,750	700	3,400	1,250	350	4,600
3d pail milk.....	2,750	1,500	1,900	3,550	2,400	3,800
4th pail milk.....		3,400		2,300		1,150
						5,800
						900
						8,300
<i>May 28, 1918</i>						
Rinsings of teat cups.....	8	0	4	10	12	
1st pail milk.....	1,800	1,300	1,750	1,000	500	
2d pail milk.....	300	900	1,000	1,100	350	
3d pail milk.....	1,250	500	4,500	9,900	300	
4th pail milk.....	2,750	1,100	1,850	250	650	
5th pail milk.....	3,550	500	3,600	1,100	3,800	
6th pail milk.....	1,950		1,050	850	350	
7th pail milk.....	6,400		2,550	500		
<i>May 31, 1919</i>						
Risings of teat cups.....	15	7	6	4	22	
1st pail milk.....	1,250	3,200	2,100	1,050	1,550	
2d pail milk.....	4,950			750	400	
3d pail milk.....	1,000	1,350	2,500	1,850	1,000	
4th pail milk.....	2,000	500	3,800	650	1,400	
5th pail milk.....	2,800	2,700	3,100	1,000		
<i>June 5, 1919</i>						
Rinsings of teat cups.....	14	4	1	7	25	
1st pail milk.....	900	1,600	1,000	427	2,150	1,450
2d pail milk.....	6,350	4,925	2,875	500	1,000	10,200
3d pail milk.....	350	4,350	1,700	650	850	1,100
4th pail milk.....	900	900	4,075	250		1,400
5th pail milk.....	3,825	750	2,100	700		2,050
6th pail milk.....	700	400	1,525	1,300		

ways taken from the pasteurizer vat before heating, after the night's milk eighteen hours old and the morning's milk seven hours old had been mixed in it. The counts are given in table 5

TABLE 5  
*Bacterial counts of machine milked milk on an ordinary dairy*

	NOVEMBER	DECEMBER	JANUARY 1 TO JANUARY 20, 1919
Bacteria per cubic- centimeter of milk	99,300,000	3,000,000	1,960,000
	25,300,000	3,360,000	2,630,000
	38,300,000	2,830,000	526,000
	7,460,000	1,146,000	2,930,000
	9,700,000	6,640,000	1,300,000
	4,500,000	3,130,000	2,700,000
	2,830,000	12,960,000	3,730,000
	1,123,000	6,130,000	8,130,000
	940,000	4,300,000	5,000,000
	400,000	5,560,000	850,000
	2,700,000	236,000	1,543,000
	3,160,000	760,000	
	1,930,000	2,000,000	
	8,700,000	2,000,000	
	39,300,000	1,060,000	
	14,600,000	213,000	
	3,800,000	230,000	
	5,600,000	930,000	
	9,000,000	1,126,000	
	41,000,000	1,123,000	
	58,000,000	2,160,000	
	20,000,000	5,360,000	
	39,000,000	810,000	
	37,600,000	1,445,000	
	29,000,000	1,860,000	
	13,000,000	3,760,000	
	2,460,000	7,900,000	
	6,230,000	2,230,000	
	6,330,000	1,600,000	
	1,360,000	1,800,000	

and show excessive numbers of organisms on nearly all the days during this period.

The excessive bacterial count of the milk caused one of us (S.) to visit the ranch in January, 1919. An inspection of the rubber parts of the milking machines showed milk residue where

the milk came in contact with them in the process of milking. The chlorine solution for the rubber parts did not seem to be freshly made up or made according to direction, although the dairyman insisted that he had followed directions in making and using it.

TABLE 6

*Bacterial counts of machine milked milk after instructions in cleaning and steaming tinware had been given*

	MONTH BEGINNING		
	January 21	February 1	March 3
Bacteria per cubic centimeter of milk	250,000	1,416,000	8,160,000
	1,180,000	1,580,000	7,630,000
	1,130,000	4,280,000	6,300,000
		4,360,000	6,600,000
		4,130,000	2,760,000
		4,800,000	
		7,130,000	
		14,730,000	
		4,000,000	
		4,960,000	
		7,560,000	
		8,100,000	
		6,130,000	
		6,200,000	
		8,530,000	
		19,600,000	
		22,600,000	
		1,300,000	
		1,030,000	
		9,000,000	

The tinware was in a poor state of repair, especially the large receiving tank, in the bottom seam of which a small leak had been stopped with soap. The insides of the utensils were greasy to the touch.

The tinware was soldered, fresh chlorine solution mixed up for the machine parts, and methods improved in a general way. This resulted in little improvement in the quality of the milk as can be observed by referring to the bacterial counts obtained from January 21 to March 10, given in table 6.

The dairyman was then requested to put in a sterilizer tank similar to the one in use at dairies "A" and "B." The rubber parts of the machines were to be put in once daily and the temperature brought to 180°F. for fifteen to thirty minutes. It was thought that sterilizing in this way once a day would reduce the count so that the milk would meet the bacterial requirements

TABLE 7

*Bacterial counts of machine drawn milk after rubber parts were sterilized by heat*

	MARCH 11	APRIL 1	MAY 1	JUNE 2
Bacteria per cubic centi- meter of milk	200,000	150,000	180,000	230,000
	160,000	200,000	120,000	160,000
	106,000	260,000	96,000	140,000
	210,000	180,000	52,000	170,000
	216,000	200,000	100,000	110,000
	150,000	207,000	230,000	190,000
	250,000	83,000	167,000	210,000
	155,000	130,000	190,000	220,000
	150,000	300,000	120,000	207,000
	340,000	200,000	110,000	205,000
	220,000	219,000	190,000	187,000
	320,000	150,000	210,000	211,000
	300,000	170,000		173,000
	87,000	230,000		198,000
	160,000	203,000		215,000
	150,000	190,000		240,000
		160,000		190,000
		79,000		200,000
				213,000
				184,000

\* June hot spell.

for grade "A" raw, to be pasteurized, which in this state is 200,000 per cubic centimeter. The tank was installed and operated the first time March 10, 1919, and the immediate effect of this method of heat sterilization of milking machine parts can be observed by referring to table 7.

#### UDDER TROUBLES

We have no positive evidence of sterilization of milking machine parts reducing udder troubles and what we have to offer is in the nature of negative evidence. It is a well known fact



that pyogenic cocci and some members of the colon group are the organisms largely responsible for mammitis. These organisms are widespread in nature. Many outbreaks of mammitis have developed in dairies where machine milking has been done and the fact that udder troubles had become more frequent than was the case with hand milking, caused many dairymen to discard the milking machine after a few months or years of use. The development of udder troubles has resulted in investigation by the manufacturers of milking machines, their selling agents, milk inspectors and others interested. The universal findings in such investigations have been an unsanitary condition of the teat cups and rubber tubing. While the same conditions undoubtedly do occur on many ranches without seeming to cause udder troubles, the fact remains that several hundred cows under our observation have now been milked with machines properly sterilized for from one to several years and no increased udder troubles have developed. Practically sterile teat cups may, therefore, be an insurance against this potential danger.

#### CONCLUSIONS

1. Heat sterilization is the only way to successfully sterilize milking machine rubber parts under ordinary ranch conditions.
2. It is a practical procedure from the time involved and the wear and tear on parts.
3. Where it has been regularly done no increased trouble with mammitis has ever developed as a result of installing machines.
4. By this means as low a bacterial count milk can be produced with milking machines as by hand milking.
5. Milk can be produced to meet any reasonable bacterial grading system.
6. No chemical solution has been found to successfully accomplish these results under practical conditions.
7. Its general application will greatly reduce the discarding of milking machines after they have been installed.

## REFERENCES

- (1) HARRISON, F. C. Machine drawn milk versus hand drawn milk. Some bacteriological considerations. 23d Annual Report of the Ontario Agricultural College and Experimental Farm, 1897, pp. 128-132.
- (2) BORDAS, F., AND DE RACZKOWSKI: De la traite mecanique, dans l'industrie laitiere. Paris Academie des Sciences Comptes Residus, 1902, 135, pp. 371-372.
- (3) STOCKING, W. A.: Bacteriological studies of a milking machine. U. S. Dept. of Agriculture Bulletin 92, pt. 2, 1907.
- (4) ERF, OSCAR: Milking machines. Kansas Agricultural Exp. Sta. Bulletin 140, 1906.
- (5) STOCKING, W. A., AND MASON, C. J.: Milking machines. Effect upon quality of milk. Connecticut Ag. Exp. Sta. Bulletin 47, 1907, pt. 1.
- (6) HASTINGS, E. A., AND HOFFMAN, CONRAD: Bacterial content of machine drawn and hand drawn milk. Wisconsin Ag. Exp. Sta., 24th Annual Report, 1906-1907.
- (7) MEEK, ELIZABETH B.: Bacterial efficiency of the milking machine. Penn. Ag. Exp. Sta. Annual Report, 1907-1908, p. 146.
- (8) BRAINARD, W. K.: The production of clean and sanitary milk. Virginia Ag. Exp. Sta. Bulletin 185, 1909.
- (9) HARDING, H. A., WILSON, J. K., AND SMITH, G. A.: Milking machines. Effect of method of handling on the germ content of the milk. New York Ag. Exp. Sta. Bulletin 317, 1909.
- (10) WING, LOIS W.: Milking machines: their sterilization and their efficiency in producing clean milk. Cornell Ag. Exp. Sta. Circular 18, 1913.
- (11) RUEHLE, L. A., BREED, R. S., AND SMITH, G. A.: Milking machines. III. As a source of bacteria in milk. IV. Methods of maintaining in a bacteria free condition. New York Ag. Exp. Sta. Bulletin 450, 1918.
- (12) HAECKER, A. L., AND LITTLE, E. M.: Milking machines. Nebraska Ag. Exp. Sta. Bulletin 108, 1908.
- (13) EDWARDS, S. F.: Bacteria and the Burrell Lawrence Kennedy milking machines. Ontario Dept. of Ag. Bulletin 159, 1907.

# THE DEVELOPMENT OF ACIDITY IN MILK

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The development of acidity in milk is a biological phenomenon which offers many very interesting but obscure problems for those to solve who are interested in scientific dairying.

Milk fresh from the cow has an amphoteric action on litmus papers but is distinctly acid to phenolphthalein solution. The acidity of milk is usually estimated, using 10 cc. of undiluted milk, by titration with sodium hydroxide solution with phenolphthalein as indicator, and the acidity is then calculated as lactic acid. By this means Koning has shown that the acidity of fresh milk from individual cows varies from 0.10 to 0.21 per cent (1). This agrees very well with the work of other investigators; but on the question of the *average* acidity of fresh milk writers disagree a little; it varies apparently with locality and other factors.

The cause of the natural acidity of milk has been the subject of some experiment. It is evidently not due to lactic acid (2).

Commercial milk in Cardiff has usually an acidity between 0.16 and 0.20 per cent and it may contain from 20,000 to 1,500,000 bacteria per cubic centimeter (counted on ordinary agar plates after three days at blood heat).

When milk is retained for some time the acidity increases due to the production of acids from the milk sugar by bacteria. This increase does not always immediately become apparent. The milk may show no increase of acidity for a period of time which has been called the "incubation phase." This incubation phase lasts longer the lower the temperature and seems to be associated in some way with a "bactericidal phase" during which the initial bacteria, or some of them, decrease in number. The acidity of the milk may actually decrease for a time (3).

Finally, then, the acidity increases and when it reaches about 0.3 per cent the milk will curdle on boiling. When it reaches

somewhere between 0.60 and 0.86 per cent the milk coagulates spontaneously.

By and by the acidity of the milk reaches a maximum, which varies according to the temperature at which the milk has been held; then the acidity gradually decreases. These changes in the acidity of the milk must be correlated with the development of bacteria in it and the biology of this matter is extremely complicated. First one type of bacteria reaches its maximum numbers and then another and we have thus a sort of rotation of germ crops in the milk.

The change in the acidity of Cardiff milks retained at three different temperatures are shown in the accompanying graphs.

The milks were placed in sterilized Erlenmeyer flasks plugged with sterile cotton wool and from week to week portions were withdrawn and titrated in the usual way. After the milk curdled instead of measuring it with a pipette, a 10 cc. glass cylinder was used which was then washed out with a *small* quantity of water into the titration flask.

In cold storage it will be seen that the acidity increases somewhat slowly and that the maximum attained is just about 1 per cent. At blood heat the acidity increases very much quicker and may attain as much as 3 per cent in one week. The lowest acidity reached in one week at this temperature was 1.6 per cent. At room temperature the acidity increases at an intermediate pace but the maximum attained varies very greatly, anything from 1 per cent to over 2 per cent. The explanation of these results lies in the type of germ which is encouraged.

The principal acid producing bacteria in milk may be conveniently divided into four groups (Löhns) as follows:

1. *The lactic streptococci group.* These are short rods, or oval cocci in pairs or in chains. They produce up to 1 per cent of acidity in milk. Their optimum temperature varies enormously but is usually about 82°F. Some grow better at room temperature than at blood heat while the contrary may be the case. They grow slowly in cold storage. They constitute the ordinary starters used in this country for ripening cream to make butter, or ripening milk to make cheese. They generally constitute

about 99 per cent of the total bacteria in milk which has just curdled at room temperature, in well ripened cream, or in green cheese a few days old. They curdle milk producing a homogeneous curd with a pleasant acid taste. They produce tiny little colonies on solid culture media such as litmus lactose agar. acidification is greater in the depths of the milk culture showing that they are somewhat anaerobic. A good strong culture of these germs seeded into pasteurized milk should produce an acidity of 1 per cent in twelve hours at 72°F. (with a 1 per cent inoculation).

2. *The intestinal lactic bacteria.* These originate from excrement and *B. coli* belongs to this group. They curdle milk giving a curd with an unpleasant sour smell, and the curd is pitted with gas holes. They are a cause of gassy curd or blown cheese. They spoil both cheese and butter. They are commonly over-numerous in milk which has been ripened without the aid of a good starter. A culture of *B. coli* here gave a maximum acidity of 0.75 per cent at room temperature or at blood heat. It grew slowly in cold storage. They are short rods and *B. coli* is motile; the other members of the group are usually non-motile. They are always objectionable but tend to be outgrown by members of groups 1 and 3 which can produce a higher acidity if the conditions are suitable. They produce well marked colonies on ordinary solid media. They are somewhat aerobic and grow best about blood heat.

3. *The lactobacilli.* These are usually long thin rods. They are used as starters in making the Emmenthal-Gruyère type of cheese, and also Grana (Parmesan) cheese. To this group belongs the well known *Bacillus bulgaricus*. Their properties vary. *B. bulgaricus* from "lactigen" produces very high acidities at blood heat (3 to 3.5 per cent). They do not grow on ordinary solid media. Their optimum temperature is usually well above blood heat. A 1 per cent inoculation of "lactigen" into pasteurized milk will produce about 1.2 per cent of acidity in twelve hours at 98°F.

4. *The lactic micrococci.* This is a somewhat indefinite group of acid producing bacteria which are principally found in milk that has been held in cold storage. They have been found

inside the udders of cows so do not necessarily get into milk from dust or dirt. They have been found in butter and cheese and their optimum temperature is said to be low.

All these four groups of lactic bacteria are found in commercial milks and we are now able to see why milk kept at blood heat develops so much acidity; it is because the lactobacilli develop quickly and strongly. They do not develop in cold storage and they may or may not develop at room temperature. Their minimum temperature is usually supposed to be about 68°F.; but it varies with different strains (4).

Thus three lots of milk, each containing lactobacilli as shown by the blood heat incubations, were kept at room temperature and were subject to the same temperature variations, yet the maximum acidities attained varied greatly as the following data show:

*Commercial milks held at room temperature*

	A	B	C
	Bacteria per cubic centimeter.		
	800,000	250,000	580,000
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Acidity at start.....	0.19	0.16	0.18
Acidity in 2 days.....	0.86	0.86	0.86
Acidity in 1 week.....	1.00	1.07	1.00
Acidity in 2 weeks.....	1.03	0.97	1.09
Acidity in 3 weeks.....	1.12	1.30	1.52
Acidity in 4 weeks.....	1.11	1.16	1.80
Acidity in 5 weeks.....	0.88	1.12	2.20
Acidity in 7 weeks.....	0.70	0.65	

Apparently the lactobacilli did not develop in A, doubtful in B, and they certainly did in C. When the acidity of milk exceeds 1.25 per cent it can usually be proven that lactobacilli are present (5). Other maximum acidities obtained at room temperature have been 1.17 per cent, 1.40 per cent and 2.01 per cent.

From time to time the aging milks were inoculated at the rate of 1 per cent into flasks of sterilized milk and held at 98°F. The subcultures from milk kept at 98°F. always attain a high degree of acidity:

NUMBER	ORIGINAL AT 98° F.		SUBCULTURE AT 98° F.	
	Age	Acidity	Age	Acidity
	<i>days</i>	<i>per cent</i>	<i>days</i>	<i>per cent</i>
1	14	2.66	5	1.94
2	14	3.19	5	1.89
3	35	2.90	7	1.73
4	105	0.88	7	1.98

In each case the subcultures developed over 1.7 per cent of acidity and then the acidity rapidly fell. Microscopic examination showed the presence of lactobacilli and yeasts. Ordinary agar plates grown at room temperature from one of the subcultures showed only yeasts. The reduction of the acidity was accordingly attributed to the yeasts. The odor of the subcultures was alcoholic, or aromatic suggesting esters, or sometimes cheesy. The originals often smelt malty. The most interesting subculture was that made from the milk retained fifteen weeks at 98°F. The acidity had reached 3.2 per cent on the sixth week but fell to 0.88 per cent in the fifteenth week. Nevertheless the subculture then made showed that the lactobacilli were still alive in the milk. One of these subcultures containing yeasts and lactobacilli gave a badly blown cheese curd smelling strongly of alcohol (6).

Subcultures from milks at room temperature also gave interesting results:

NUMBER	ORIGINAL AT ROOM TEMPERATURE		SUBCULTURE AT 98° F.	
	Age	Acidity	Age	Acidity
	<i>days</i>	<i>per cent</i>	<i>days</i>	<i>per cent</i>
1	14	0.99		Never above 1
2	14	0.99		Never above 1
3	35	1.34	12	1.41
4	105	0.92	7	1.65

Here again the most interesting culture is the fourth where the milk held at room temperature had reached an acidity of 2.01 per cent in six weeks but decreased to 0.92 per cent on the

fifteenth week; yet the subculture showed that the lactobacilli were still alive and active.

Subcultures made from milks held in cold storage for seven to fifteen weeks never at any time showed over 1.26 per cent of acidity when incubated at blood heat for seven days, and two of them kept till fourteen days showed then a decreasing acidity.

These results indicated that lactobacilli are invariably present in Cardiff milks, that they may or may not develop at room temperature, and that when they do develop the high acidity they produce favors the growth of yeasts which subsequently reduce the acidity. This agrees with the results obtained by Orla Jensen, Rosengren and others on the bacteriology of butters; high acidities favor the yeasts which subsequently deteriorate the butter.

A sample of milk kept at room temperature for five weeks showed a maximum of 1.17 per cent of acidity and on the ninth week the acidity fell to 0.92 per cent. Whey agar plates then made from the milk were rapidly overgrown (two days) at 98°F. by oval spore formers; while gelatine plates were liquefied. Similar plates are often made from boiled hay. The sample of milk was then thoroughly pasteurized to kill yeasts, moulds and non-sporing bacteria and held at 98°F. for a week. During that time the acidity dropped from 0.92 to 0.60 per cent. The reduction of the acidity in this case was not due to yeasts but to spore-forming rods which were found to be capable of producing a badly blown cheese curd (7). Subcultures in liquid media showed the presence of long rods with an undulating motility. They produced 1.1 per cent of acidity in milk at blood heat in three weeks.

Other organisms sometimes taking part in the final reduction of the acidities were moulds—*Oidium lactis*, *Penicillium*, etc. In one case a milk held four weeks at blood heat went into a solid lump with moulds so that no further acidity estimations were possible.

The four groups of lactic bacteria already referred to do not form spores and so do not survive pasteurization. When milk is pasteurized spore-forming rods survive. When held subse-



quently at almost any temperature within reasonable limits these milks finally curdle and at that time may or may not show an increase of acidity compared with the fresh milk. The curd, however, begins to digest and the acidity of the milk will then rise.

Three samples of pasteurized milk were kept for three weeks in cold storage yet the acidity did not exceed 0.18 per cent in any case and the milks did not curdle on boiling. Two samples of one milk were pasteurized thoroughly in boiling water and then cooled and kept at blood heat. They curdled in three days when one showed an acidity of 0.18 per cent; the acidity of the other in sixteen days when the curd was almost completely digested was 0.45 per cent. Two samples of the one milk were pasteurized, the first at 165°F. for five minutes, and the other at 210°F. for fifteen minutes. In fourteen days at blood heat the acidity of the first reached 0.83 per cent and of the other 1.03 per cent. These cultures smelt like strong cheese. Some of these spore formers can evidently produce a fair degree of acidity in milk. In raw milks they are held in check by the lactic bacteria; but if the lactobacilli do not develop, and since the streptococci die out fairly rapidly after reaching their maximum numbers, the spore formers may then get an opportunity to develop in the old milk as has already been shown.

These experiments, carried out at the King Edward VII Hospital, Cardiff, were designed to cast some light on the bacteriology of the keeping properties of butter, and of the ripening of cheese; but the subject has proved immensely complicated. They may however serve to interest others in a most interesting subject which has been rather neglected in this country.

In the University College Dairy we used ordinary starters of lactic streptococci to ripen the milk for making Cheddar cheese. On two occasions, when starter was being added to the milk at the rate of 2 per cent, experimental cheeses were made in which half the starter was replaced by a culture of *B. bulgaricus* (lactigen). The resulting truckle Cheddars when ripe differed in no way (neither in flavor nor in texture) from the control cheeses made on the same day.

A fairly complete summary of the earlier experiments on this subject will be found in Lafar's *Handbuch der Technischen Mykologie*, and Dr. Fascetti has an original article on "The principal and most recent applications of bacteriology to the dairying industry" in the *International Review of Agriculture*, of February, 1915.

## REFERENCES.

- (1) KONING: *Biologische und Biochemische Studien über Milch*, Leipzig, 1906, p. 53.
- (2) VAN SLYKE AND BOSWORTH: *New York Exper. Sta. Bull.* 37, 1914; also Koning, loc. cit.
- (3) KONING: loc. cit., p. 20.
- (4) FREUDENREICH AND THONI: *Landw. Jahr. der Schweiz*, vol. 18, p. 525; also Rogers, *Fermented milks*, U. S. Dept. Agric., Bur. Animal Ind. Bull. 319, p. 21.
- (5) HASTINGS, EVANS AND HART: *The bacteriology of Cheddar cheese*, U. S. Dept. Agric., Bur. Animal Ind. Bull. 150, p. 32, 1912.
- (6) U. S. Dept. Agric., Bull. 148, p. 13.
- (7) DAIRE: *Les microbes dans l'industrie laitière*, 1914, p. 113.

## Thomas Corneliuson

Thomas Corneliuson, dairy manufacturing specialist of the Dairy Division, U. S. Department of Agriculture, died on October 3, 1919, at Belleville, Wisconsin, after a lingering illness of six months. Mr. Corneliuson, who has long been active in dairy work throughout the United States, leaves a host of friends who will mourn this new loss to the dairy industry.

Up to the time of his illness Mr. Corneliuson was in active charge of the inspection of the large quantities of butter produced for the United States Navy, and also directed the inspection of the renovated butter produced in this country.

Mr. Corneliuson, who was born in Denmark, April 18, 1865, received his early training in creamery work in that country. After serving several years as student and buttermaker in Danish creameries he decided that the United States held greater opportunities in store for him and accordingly came to this country in 1890. While connected with creameries in this country he attended the dairy school at the University of Wisconsin, completing several short courses and serving as instructor in buttermaking.

The thoroughness of Mr. Corneliuson's work and his remarkable memory for details soon won recognition, and in 1904-5 he was made a travelling cream instructor under the auspices of the Wisconsin State Dairymen's Association. The following year he became State Creamery, Dairy and Food Inspector for the Dairy and Food Commission, Madison Wisconsin, and continued in this capacity until 1908, when he was appointed to a responsible position with the United States Dairy Division.

Besides being an expert buttermaker, Mr. Corneliuson was one of the foremost butter judges of this country and rendered great service in this capacity. Conscientious, thorough and always striving upward, Mr. Corneliuson has won many staunch friends who will deeply regret his death.

Mr. Corneliuson was buried in Glenwood Cemetery, Washington D. C., on October 11, 1919, the services being conducted by the Lafayette Lodge of F. A. A. M., and DeMolay Commandery of Knights Templar, both of Washington, D. C.—*Helmer Rabild.*

## ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION<sup>1</sup>

The annual meeting of the Dairy Science Association was opened at eleven o'clock in the Stock Yards Inn. After a few brief opening remarks by President Anderson the report on constitution was made the first order of business.

The report made by Chairman Eckles follows:

### PROPOSED CONSTITUTION FOR DAIRY SCIENCE ASSOCIATION

*Article 1.* The name of this organization shall be the American Dairy Science Association.

#### OBJECT

*Article 2.* The object of the Association shall be to advance the general welfare of the dairy industry, especially by the improvement of dairy instruction, by the stimulation of scientific research in all phases of the subject and by the improvement in methods of conducting extension work.

#### MEMBERSHIP

*Article 3.* Membership shall be of two kinds, active and associate.

*Article 4.* The following are eligible for election as active members:

(1) Any person who is formally announced by an Agricultural College, or Experiment Station or by the Dairy Division of the United States or Canadian Departments of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry.

(2) Any one filling a position of responsibility connected with the dairy industry of a professional character requiring technical scientific training.

*Article 5.* An active member ceasing active duties in connection with the dairy industry is transferred to associate membership. Any person interested in the dairy industry but not eligible under the

<sup>1</sup> Credit is due President A. C. Anderson and Secretary N. W. Hepburn for the following report of the annual meeting of the American Dairy Science Association.—J. H. FRANSEN, *Editor*.

qualifications as prescribed for active membership may be nominated for associate membership. Associate members shall be entitled to all the privileges of the Society except that of voting.

*Article 6.* Nominations for active or associate membership shall be submitted to the executive committee in written form and signed by at least three active members. Upon receiving the unanimous endorsement of the executive committee and paying the annual membership dues, the candidate shall be duly enrolled as a member of the Association.

#### OFFICERS

*Article 7.* The officers of this Association shall be president and vice-president, whose term of office shall be one year; a secretary-treasurer and a journal editor, whose term of office shall be two years. The term of office shall begin January 1.

The president, the vice-president, the secretary-treasurer and the presiding officers of divisions of the Society shall constitute the executive committee.

#### DUTIES OF OFFICERS AND MANNER OF ELECTION

*Article 8.* The duties of the officers shall be those usually pertaining to their respective offices. The executive committee shall pass upon all nominations for membership, all applications for divisions and sections of the Society. It shall have the power to fix the time and place of the annual meeting, and, shall be authorized to transact such business of the Association as demands attention while the Association is not in session.

*Article 9.* On or before November 1 the secretary shall mail to each member a blank upon which he shall be entitled to express his choice for each office to be filled. This blank shall give the names of those serving in the offices to be filled for five years preceding. All ballots shall be counted by the secretary and later verified by the president. In case no candidate has a majority by the first ballot, a second ballot shall be sent to the members giving the names of the three leading candidates for each office and the number of votes received. The candidate receiving the most votes on the second ballot shall be declared elected. In case of a tie on the second ballot the decision shall be made by the executive committee.

## ORGANIZATION OF DIVISIONS AND SECTIONS

*Article 10.* Professional groups based upon geographical considerations to be known as divisions of the Society and to be organized by members of the Association may be authorized by the executive committee when such action shall seem expedient. The officers of the division shall be a chairman and such other officers as are provided by the division. The presiding officers of division shall be ex-officio vice-presidents of the Society.

The divisions shall have the right to make by-laws for its own government and which shall not be inconsistent with the constitution and by-laws of the Association.

Membership in divisions of the Society is open only to those regularly elected members of the Society.

Any division may raise or collect funds to be expended for its own purpose.

*Article 11.* Professional groups based upon specialized interests to be known as sections of the Society and to be formed by not less than ten active members may be authorized by the executive committee when considered for the best interests of the Association.

Such sections may elect their own officers and make any rules for their own guidance not inconsistent with the constitution and by-laws of the Association.

## AMENDMENTS

*Article 12.* This constitution may be amended by a two-thirds vote at any regular meeting of the Association; provided the proposed amendments have been submitted to the executive committee in writing not less than thirty days previous to the meeting at which the vote is taken; and provided the proposed amendment is approved by a majority of the executive committee.

*Article 13.* The executive committee may at its discretion, submit proposed amendments which have received the approval of the committee to the members of the Association for vote by mail. An affirmative vote of two-thirds of all voting and which shall be not less than a majority of the membership shall be necessary for approval.

## BY-LAWS

*Article 1.* The membership dues shall be \$3 a year for both active and associate members, payable January 1 each year.

*Article 2.* The JOURNAL OF DAIRY SCIENCE shall not be sent to any member whose dues are not paid by April 11 of the year for which membership is held.

*Article 3.* Any member in arrears for dues for more than one year shall thereby cancel membership but may be restored to membership without any action by the Society by payment of all arrears including dues for the current year.

*Article 4.* The time and place of the meetings of the Society shall be fixed by the executive committee.

*Article 5.* A quorum at any meeting for the transaction of business shall consist of not less than 10 per cent of the active members.

*Article 6.* These by-laws may be amended at any regularly called meeting by a two-thirds vote of those present.

*Action on report on constitution*

Moved by Eckles, seconded by Cooper that the amendments to the constitution be adopted as read.

Moved by Wing, seconded by Stocking that membership plan no. II be adopted.

Above motion put to vote. Carried.

Moved by Eckles that present officers hold over to January 1. Carried.

Next the report on relation to Breed Associations was called for. Professor Eckles, chairman of this committee, made the report which follows:

REPORT BY COMMITTEE ON RELATION TO BREED ASSOCIATIONS

WHEREAS, the Superintendent of Advanced Registry of the Holstein Friesian Association of America, in reviewing the tests of semi-official records as reported by the Experiment Stations, has adopted a practice by which tests for individual months are rejected and averages for the preceding and succeeding months substituted, thereby either increasing or decreasing the particular record in question.

*Be it resolved* that this organization go on record as being strongly opposed to any practice by which tests are reported by the Experiment Stations may be adjusted by the Superintendent of Advanced Registry and that we urge the adoption of a system of automatic verification tests in connection with semi-official records, thereby increasing the official portion of the records rather than decreasing it as is the case under the present system.

It is recommended that in order to safeguard Experiment Stations and Agricultural Colleges from legal complications in vouching for Advanced Registry Testing that a statement setting forth the limits of the responsibility assumed should be stamped upon each report at the time the signatures of the Superintendent of Official Testing is affixed.

That Advanced Registry Testing should impose no financial burden upon the institution supervising such testing and that such institutions are justified in appropriating from the breeder for whom their testing is done the full expenses incurred including the full expenses of the supervisor.

That the Dairy Science Association ask the Holstein Friesian Association to either discontinue admission to the advanced registry on a seven day test or to extend that time which must elapse after freshening before the test is begun.

Moved by Eckles, seconded by Prucha that Part I of this report be adopted. It is understood that the committee will furnish the wording. Action. Carried.

*Section II.* Moved by Regan, seconded by Gamble that Section II be adopted as read. Action. Carried.

Section II was to be sent with proper explanations by the committee to Deans of Colleges and Heads of Departments.

*Section III.* Section III caused considerable discussion during which adjournment was made for lunch.

Meeting was called to order at 1.30 and discussion resumed on Section III of Eckles' report.

It was moved by Regan, seconded by Brant that the committee request the Association of Agricultural Colleges and Experiment Stations to be relieved of the responsibility of conducting all tests on all breeds of less than 300 days duration after October 1, 1920.



*Section IV.* Moved and seconded that the resolutions apply to all breeds. Carried.

Moved by White, seconded by Harding, that the committee on relation to breed associations be informed to present this matter to the Association of Agricultural Colleges and Experiment Stations.

After disposing of the report on relation to breed associations a report for the committee on milk quality was made by the chairman, Dr. Harding.

Mr. Gamble moved that this Association go on record favoring standardization of milk under proper supervision. (The composition to be stated on the label).

Remarks were made by Kelley of Washington and Professor Troy of Cornell. Action. Carried.

Dr. Harding's report follows:

#### STANDARDIZATION OF MILK

A part of the fascination of city milk problems arises from the fact that something new is always developing.

You are familiar with the minimum legal limits of milk composition which are in force in every state. You know that this form of legal enactment came into vogue when it was supposed that there was some natural limit below which milk would not go unless it was adulterated. You know that this idea is erroneous and that today a very considerable fraction of the milk of the country as it comes from the cow is illegal from the standpoint of these minimum legal standards. The most common deficiency is in "solids not fat," but occasionally one finds entire herds which are below the legal limits in both "fat" and "solids not fat." I think everyone conversant with the facts agrees that this illegal milk is an entirely wholesome and desirable food, its sole disadvantage being that it is not worth as much for food as an equal volume of a richer milk. Naturally, the milk producers who are selling this wholesome food just as it comes from the cow resent being branded as criminals. There has, accordingly, arisen on the part of the milk producers a widespread dissatisfaction with these dairy laws.

Following the unprecedented high price of milk the consumers are beginning to inquire regarding the food value furnished by milk. It develops that under the guise of milk they are liable to obtain an

article carrying anywhere from 3 per cent of fat and furnishing 546 calories per quart to one carrying 5 per cent of fat and furnishing 777 calories of energy per quart. They are interested in knowing a little more regarding the real value of the milk which they are buying at present high prices.

Until recently the milk dealers bought milk on the basis of weight and the composition was not a serious matter to them so long as it was above legal limits. Now milk is practically all purchased on the basis of fat content. While formerly the fat content did not enter into the cost price, now a variation of 0.1 per cent in the fat content of the milk purchased by a single company may mean as much as \$100,000 per year.

These movements among the producers, handlers, and consumers of milk all have a common influence toward demolishing the old conception of milk as a substance of uncertain composition, and call for the recognition of milk or milks of definite composition to fit definite situations. In short, we are rapidly approaching the standardization of milk.

Standardization is a natural part of milk quality but because it has a number of angles and relationships I have hesitated to assume that it was a problem to be taken over by the committee on milk quality. I did, however, urge that the officers of the Association give the matter of milk standardization and milk standards consideration at the present meeting.

Your president has directed that it be taken up with the committee on milk quality, but as his instructions to this end did not reach me until last Saturday, time has not permitted consideration by the committee.

In attempting to get justice done, the milk producers who chance to have low testing cows, it seems fairly clear that little can be accomplished by attempting to have the present legal limits slightly lowered. Such action is not a solution for the real difficulty and brings one into sharp conflict with the representatives of the consumer who insists that the standards are now too low. Again, in the case of milk entering into interstate commerce, little can be accomplished unless a change is made in the laws of both states, as well as the federal regulations. This is manifestly a difficult undertaking.

Recognizing the magnitude both of the problem and of the difficulties involved, it has seemed best to strive for a concerted action on the part of the various interests involved. To this end the matter

is coming up in the various national bodies interested and later there will undoubtedly develop some satisfactory solution.

It seems fairly plain that if milk is sold for what it actually is, the situation will be fair to all concerned. It would seem that in the case of city milk this would lead to standardization and sale with an accompanying statement of the fat content and such other items as may be deemed necessary.

If this Association wishes to be a force in working out a better basis for milk standards than that at present in vogue, your committee on milk quality would like instruction or suggestions as to its future action with regard to this matter.

At this juncture Professor Van Norman came into the meeting and was asked to make a talk.

Professor Van Norman gave a résumé of the development of the dairy show and of the dairy association, stating something of the position of influence that it should occupy. In the course of his remarks Professor Van Norman presented his ideas of a world's dairy congress and urged the appointment of a committee to coöperate with other agencies to work on such a program.

Moved, seconded, and carried that a committee be appointed by the president to work with other agencies toward the end of making out plans for a world dairy congress.

Report called for on testing butter for fat.

A short verbal report was made by Professor Troy. Extensive remarks on this subject were made by Professors Bouska and Hepburn.

No action taken.

Report on Legal Standards for Butter presented by Professor Mortensen, Chairman. The report follows:

#### REPORT ON LEGAL STANDARDS FOR BUTTER

As this committee had already completed its most important work a couple of years ago, there is only a brief report to present at this time.

The question of butter standards is a very important one at this time. There seems to be a general tendency on the part of the public at present

to regulate the various industries and to demand that standards be fixed, especially for such important foods as dairy products. The members of the American Dairy Science Association welcomes these reforms. We consider that it is fair to consumers, producers, and manufacturers to adopt standards, if such are formulated, so they are fair to all concerned. No standard will be fair to the consumer or to the American dairyman or manufacturer, unless it is high enough so it will protect the quality of the American butter, but it is just as unfair to establish a standard so high so that it will be impossible for the American dairyman to comply therewith during certain part of the year.

We commend the work of the American manufacturers of dairy products. They are progressive and are not slow in adopting the modern methods in the process of manufacture, and wherever we go we usually find modern creameries operated by up-to-date butter makers. Compare today's creamery butter with butter made on the farm, and there is a remarkable contrast. Analysis made of farm dairy butter indicates that there is no uniformity in the product, the composition varying much with local conditions. It is to be regretted that only a very limited amount of data was available; more work would have been done at the Iowa State College had there not been a shortage of men in the Dairy Department.

The following analyses were made by the Dairy Department of Iowa State College of Iowa farm butter during the month of June, 1919:

DATE	MOISTURE	SALT	FAT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
June 19.....	12.5	2.49	81.75
June 19.....	22.1	2.23	75.25
June 19.....	16.85	3.47	79.00
June 19.....	18.75	2.85	75.75
June 19.....	17.35	1.48	76.25
June 19.....	15.0	0.69	82.25
June 20.....	10.9	6.13	80.00
June 20.....	14.45	0.80	82.75

The following analyses were made by Mr. W. B. White of Cornell University:

H <sub>2</sub> O	NaCl	CASEIN
10.63	6.25	1.36
11.22	3.92	0.99
15.18	2.60	0.86
10.74	3.94	1.19
8.02	4.27	1.41
9.86	5.06	0.73
8.84	7.00	1.44
12.76	4.86	1.52
11.29	1.91	1.08
13.04	2.24	0.81
13.83	3.42	1.23
14.65	2.34	0.93
12.54	4.58	0.83

Moved by Professor Mortensen, seconded by Professor Lee that the report be adopted as read. Carried.

Moved we adjourn to meet at 7 p.m., Hotel La Salle, for our annual banquet. Carried.

A delightful banquet was served in the East Room, Hotel La Salle with about seventy in attendance. This was the largest banquet ever held by the Association. The principal address of the evening was given by Dean E. Davenport, Dean of the College of Agriculture, Urbana, Illinois. Following Dean Davenport's address the association listened to an informal talk relative to the future of the JOURNAL OF DAIRY SCIENCE by Professor Frandsen, editor of the JOURNAL.

Professor Erf, Chairman of the Committee on Cost of Production, then made a report which follows:

#### REPORT OF COMMITTEE ON COST OF PRODUCTION

A further development of the dairy business and the maintenance of an adequate milk supply is positively necessary for the development of man. Since milk is one of the most important foods and for which there is no substitute for developing health in children and maintaining the health of the adult, it is of the utmost importance that the supply be kept uniform and the price constantly at a level that will insure a supply of milk sufficient to provide for the demands of the increasing population and for the growing consumption that inevitably follows when people are educated as to its food value.

## ECONOMICS OF MILK

If prices are too high, consumers are required to pay so much for milk that the consumption is consequently curtailed; production is unduly stimulated on the farm and whenever possible farmers will enter into production more extensively and will cease to supply the normal supply of other foods. This inevitably results in injury to the farmer who has made his plans with the idea in mind that the high price will continue.

On the other hand, it is possible for a time to hold the price so low that production will be curtailed. If the range between cost of production and the price received for a product is very great then a reaction usually follows in which the difference is in the opposite direction. The full effects of low prices are not immediately apparent, due to the fact that no costs are available and by the time that the haphazard producer realized the fact that he is suffering a loss, the intelligent dairyman has been forced out of business.

As a result, the dairy business will be demoralized to a considerable extent. Low prices result in the raising of a decreased number of heifer calves and consequently prevent the normal extension of the dairy business.

In the past the price of milk has been quoted in advance, for periods of a month or more. No feasible plan has been developed for selling milk on the daily market in the way in which grains are sold, and on account of the nature of the commodity, such markets are not likely to be developed. Since prices are quoted in advance, it is impossible to exactly foretell the supply or demand. Obviously the price of milk should be adequate to maintain a constant supply. The maintenance of the proper number of cows is more important than an excess or shortage of supply in any particular period, which may be too high or too low on account of the character of the season or other conditions.

The safest basis for forming a judgment as to what prices are necessary in order to maintain the number of cows that are necessary to furnish the required amount of milk continuously, is cost of production and we recommend that the price be made based upon costs for short time periods. Experience has shown that the monthly basis is the best as in no case should the contract be made to exceed three months.

## COST OF PRODUCTION

It has been stated in the past that in discussing cost of production, the object in view should be clearly defined. There should be a distinction between the cost studies which have for their purpose arriving at the relative profitableness, with a view to obtaining a basis for choice on the part of the farmer, who has such an alternative, and those who have for their purpose the determining of what constitutes a fair price.

Within the past few years these conditions have brought on a feeling of mistrust on the part of a large number of milk commissions, food administrations and other public officials, in regard to the methods of figuring costs. While from the accountant's standpoint, farm cost studies may be theoretically more correct the practicalness of obtaining the figures frequently stands in the way. After all, the allocating of the various items might be incorrectly estimated unless the work is done by a competent man who can get true estimates and place a true proportional value upon each item. We therefore recommend that system of accounting be used, which may be termed "the departmental system" in which the dairy constitutes one department. For our purpose this should be given the chief consideration; although if other departmental accounts can be determined in addition, it will be exceedingly helpful in determining the relative incomes. For it might give the consumer an opportunity to see that the farmer while profiteering in one department was losing in another.

There are several methods by which dairy production costs may be determined: (1) the individual cow costs or the herd costs. While it is possible to separate herd costs from individual cow costs in some items, as a rule individual cow costs is the better method of securing cost of production.

These facts have been brought out in the cow testing associations and need not be mentioned here. It is for this reason that the cow testing associations have been converted into cost accounting associations, the latter differing from the former in that it includes labor as well as other costs in determining the profitableness of the cow and of the herd. Therefore, cow testing associations and cost associations might be inaugurated, and has been done in some instances. The combination makes a very practical and profitable method of obtaining accurate figures, and we submit it to you for your consideration, as to whether you will endorse this method of dairy cost accounting.

In figuring the cost of milk production with the individual cow cost accounting system, three accounts must be taken into consideration, namely: (1) the producing herd account; (2) the young stock account; and (3) when a bull is kept, a bull account.

The net cost of the young stock determines the proper valuation at which the heifers are placed in the inventory of the producing herd. In this case, when a calf is four days old, credit is given to the producing herd account and the calf is turned over into the young stock account. When this heifer grows to maturity and is producing milk the heifer is turned over into the producing herd account and given credit in the young stock account. In the same manner the bull service is charged to the cow. The net cost of keeping a bull divided by the number of cows determined the breeding charges per cow.

For the purpose of convenience and relative importance the selling price of milk should be determined at three points: (1) its value upon the farm, (2) its value at the country plant, and (3) its value in the city. Prices are determined by comparisons and it is unfair to make unequal comparisons, as is so frequently done under present conditions. Milk delivered in the smaller country towns may be delivered at a comparatively low price but still net the farmer more on the farm than when shipped to a large city. This oftentimes does the smaller city a great injustice; and the opposite may likewise be true. We, therefore, suggest that in cost accounting systems, the selling price be considered at two points at least—cost on the farm, the cost in the city, and whenever a county plant is used, a third cost should be given.

#### THE PRODUCING HERD ACCOUNT

The producing herd account is divided into two parts, the items relating to receipts and the items relating to expenditures. The expenditures of cost, minus the receipts or credits, will give the net cost of the milk sold.

1. *Receipts.* Under receipts are included milk and other dairy products, dairy products used upon the farm, calves, manure, hides, feed bags and other credits.

2. *Expenditures.* Expenditures are divided under three general heads—feed, labor and other expenses.



DETAILED DESCRIPTION OF PRESENTING DATA ON THE COST OF  
MILK PRODUCTION

*Milk and other dairy products.* Credit receipts for all sales. Note: Care must be taken that the credit is properly construed, depending upon the point at which the credits are paid. In most cases the credits are given at the point of distribution or in the city; however, in some cases they are paid at a point known as the country plant or receiving station. In a few cases they are paid at the farm.

*Dairy products used at the farm.* Credit the quantity of dairy products used at the farm. The price of milk at the farm, not including transportation, should be charged.

*Calf.* The value of the calf when four days of age should be credited to the herd account. Note: If a grade calf, the value should be the current price based upon slaughter value, depending upon the size of the calf and the breed, plus the value of the record of performance of the ancestors, if such records are available. If a pure bred, it should have pure bred value, depending upon the record of performance of the ancestors. In case the calf dies after it is one day old, credit for the hides should be given.

*Manure.* Credits for manure, according to the office of the Farm Management of the United States Department of Agriculture, manure ordinarily should be credited at \$1.50 per ton, for the tonnage actually recovered, but owing to the increased price of commercial fertilizers during the war, manure may be charged at perhaps \$2 per ton which would mean \$2.75 in the field. However, no specific amount should be stated in this report as its value depends to a great extent upon the locality. The average cow voids about 56 pounds of manure a day and the amount recovered is that which is usually accumulated in the stables and in the yards.

Professor White of the United States Department, Chairman of the Committee on Students Judging Contest, made the following report which was adopted as read.

REPORT OF THE COMMITTEE ON METHODS OF CONDUCTING A  
STUDENTS' DAIRY PRODUCTS JUDGING CONTEST

Since the last contest was held two years ago, there has been under discussion the advisability of making some changes in the rules, and, after discussing the matter with various members of the dairy departments concerned, the following rules and changes are recommended:

1. This contest shall not be held unless three teams shall have been entered at the time specified in these rules.

2. In order that the contestants may know the standard used by the official judges, the latter shall select two additional samples of each product representing high and low quality respectively, and shall score and criticise these samples. The score and criticisms for each package of butter, cheese and milk shall be plainly written on a card signed by the judges and attached to the sample. These standard samples shall be available for examination by the contestants immediately before the contest.

3. In addition to placing scores upon the products, each student shall write his criticism or state his reason for the score given.

4. In determining the rating of the contestants, equal rating shall be given to the score and the criticism.

5. The butter shall be scored and criticised in accordance with the system outlined in Service and Regulatory Announcement No. 51 of the Bureau of Markets, U. S. Department of Agriculture.

6. The committee is authorized to devise a method of rating the criticisms on the products and make any minor changes in the rules that seem desirable.

Professor Frandsen, Chairman of Committee on Score Cards for Dairy Products, reported as follows:

First that the committee recommend that the American Dairy Science Association officially adopt the following score cards:

<i>Cheese</i>		
		<i>points</i>
Flavor .....	45	
Texture.....	30	
Make-up.. ..	15	
Color.....	10	

<i>Score cards for butter</i>		
		<i>points</i>
Flavor.....	45	
Body.....	25	
Color .....	15	
Salt.....	10	
Package.....	5	
<hr/>		
Total.....	100	

*Score card for cream*

	<i>points</i>
Bacteria.....	35
Flavor and odor.....	25
Fat.....	20
Sediment.....	10
Acidity.....	5
Bottle and cap.....	5
Total.....	100

Relative to the score card for milk, the committee recommends that definite score cards as for milk be left open for study during the year, but in order that the dairy product coaches for the next students contest may have something definite on which to work, the committee recommends that for next year's contest the following score card be used as basis:

	<i>points</i>
Bacteria .....	35
Flavor and odor.....	25
Visible dirt.....	10
Fat.....	10
Solid, not fat.....	10
Acidity.....	5
Bottle and cap.....	5
Total.....	100

The committee does not feel warranted at this time in recommending score cards for ice cream, milk powder or condensed milk, but urges that the committee be continued and additional men be added to the committee and that during the coming year an especial study be made relative to score cards of these products.

Professor Frandsen moved that the committee's report be adopted. Motion duly seconded and carried.

Professor Lockwood, Amherst, was asked to prepare a report covering his studies of Dairy Departments of the country while he was with the DeLaval Separator Company. The report presented was adopted as read.<sup>2</sup>

<sup>2</sup> This report was published in vol. ii, no. 6 (November issue), JOURNAL OF DAIRY SCIENCE.—*Editor*.

# MOTTLES IN BUTTER—THEIR CAUSES AND PREVENTION

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*Definition of mottles.* Mottles refer to uneven color in butter, causing the appearance in the body of the butter, of deep yellow, translucent blotches interspersed by whitish, opaque dapples. Mottled butter differs from wavy or streaked butter in that in the wavy butter, the different shades of yellow appear in waves, streaks, or layers and the color in one and the same layer may be uniform or solid.

*Effect on market value.* While mottles in butter do in no way affect the flavor, keeping quality and wholesomeness of butter, and while therefore the criticism of the trade appears to be a purely superficial one, the objection to mottled butter is no less real. Butter that is otherwise of good quality and might score a good "extra," if it is mottled, clears as a "second" and is purchased on that basis by the dealer.

*Experimental results of other investigators.* Like other butter defects not related to flavor and keeping quality, so has this color defect—mottles—failed to be subjected to extensive scientific experimental investigation by the majority of dairy investigators. And the conclusions at which those workers arrived who did concentrate their efforts on this defect, in a large measure fell short of a satisfactory solution of the causes and practical method for the prevention of mottled butter. The dairy literature shows research on mottled butter by Storch (1), Van Slyke and Hart (2) and by Sammis and Lee (3).

Storch's work, done prior to the year 1897, represents the most comprehensive experimental study on this subject, but some of his conclusions were entirely erroneous. He concentrated his endeavor on a study of the fat globules and butter granules. Accordingly he found that each fat globule in milk and cream is surrounded by a slimy nitrogenous film. In the process of

churning a part of this film of each globule has to be torn away in order for the globules to unite into butter granules. When uniting, a part of this film is left around each fat globule while the remainder is torn away, becoming enmeshed between and in the granules of butter. He further holds that this slimy nitrogenous film is the only part of the fat globules capable of holding water.

Storch considers that there are two kinds of water in butter. The first kind is that which is contained in this nitrogenous film of the fat globules and such portions as have been torn away. The second is the water which is gathered up by the butter granules in their process of forming, from the buttermilk, and part of which is later replaced by the water used in washing the butter. The water contained in the slimy nitrogenous film consists of exceedingly minute droplets very completely emulsified. The water that the butter granules pick up from the buttermilk is present in the form of larger droplets not so firmly held by the butter granules.

Storch found that it is these localized units of very fine droplets, emulsified in the nitrogenous slime that give mottled butter the opaque, thick, whitish dapples, and that it is the larger droplets resulting from the water which the granules pick up from buttermilk and the wash water, that give the butter the deeper yellow, clearer blotches.

Storch attributes these uneven distributions and localizations of the small and large droplets to the particular fermentation that goes on in the ripening process of cream, and he endeavors to show that a bitter milk organism produces light local opaque color when used for ripening the cream, while butter made from lactic-acid ripened cream produces a clear, deeper yellow butter. He even goes so far as to claim that when he ripened cream with starter made from the light colored blotches of mottled butter, the butter so resulting would be light in color, while when he made butter from starter inoculated with the dark, clear blotches of mottled butter, the resulting butter would be of that color, and he further states that microscopic counts of the water deoplets show a similar predomination of the large or

small droplets in the experimental butter and the original butter from which the starter was made.

Storch therefore concludes that unevenness in color is due to different fermentations which are going on in the ripening of cream, and he states that if cream is ripened in several different receptacles, even if the ripening process is started in exactly the same manner, different species of microorganisms may gain the ascendancy in the different receptacles. When this cream is churned separately into butter and the butter then is worked together, mottles are produced.

The writers fully agree with Storch in his deductions that certain ferments are capable of making lighter colored butter than others. The fact is that ferments which act on the curd in cream in such a manner as to increase its viscosity and therefore its emulsifying power tend to produce a butter in which the water-in-fat emulsion is very complete and which, owing to the great multitude of extremely finely divided water droplets, is very opaque and has a very light color. To this group of ferments belong most of the proteolytic and liquefying microorganisms, including organisms capable of making cream bitter.

That butter made from lactic-acid ripened cream has a somewhat more open body and deeper yellow, clearer color than sweet cream butter is well known. In this case the curd is acted upon in the opposite direction. The acid causes it to be precipitated and to become less viscous. Its emulsifying power is reduced. Hence a butter is produced in which the water-in-fat emulsion is less complete, the water droplets are fewer in number and larger in size and this in turn causes it to have a clearer and deeper yellow color.

But from this point on Storch's conclusions concerning the causes and prevention of mottles in commercial butter are utterly erroneous, and their application fails to solve this butter defect. Mottles in commercial butter have nothing whatever to do with the ripening process of the cream, they are exclusively caused and controlled by factors related to the salting and working of butter, as will be shown in subsequent paragraphs.

Van Slyke and Hart, as the result of an extensive experimental study of the causes of mottles concluded that mottles in butter are due, primarily, to the presence and uneven distribution of buttermilk adhering to the outer surface of the small granules, and secondarily, to the hardening and localizing effect of salt brine upon the proteid of the buttermilk thus retained in butter. They hold that the light portions of mottled butter owe their lighter color to the presence of localized proteid (usually casein lactate) and that the yellow or clear portions occur where the spaces between the butter granules are filled with clear brine and are comparatively free from casein compounds. They further state that several hours are required to complete the action of the brine upon the proteid of butter. They found by experimental analysis that the amount of proteid (casein lactate) in mottled butter is often slightly greater in the light portions than in the darker portions and conclude that this is the cause of the lighter color of the mottles.

On the basis of the above findings and conclusions Van Slyke and Hart suggest that mottles in butter can be prevented by avoiding those conditions that retain buttermilk in the butter and observing those conditions that favor the removal of buttermilk from butter granules before salting, and that the butter granules should be about the size of rice grains and should be washed twice with water at a temperature of 35° to 45°F. It is obvious from what has already been stated, and from results of the experiments described later in this discussion, that these conclusions, too, are incorrect and the application of the directions offered by these investigators has not the slightest effect on the presence or absence of mottles.

Sammis and Lee repeated a portion of Storch's investigation. They found that butterfat, freed from casein by melting and filtration, then emulsified with water and churned, produced typical mottles when the salt was not evenly distributed throughout the mass. They thus produced mottles entirely independent of the casein. Microscopic examination of such butter showed similar results as in the case of Storch's experiment. In the portions which were lighter in color, the water was present

in the form of innumerable small droplets, while in the portions that were darker, the droplets of water were much larger. No counts nor measurements of the water droplets were given. These investigators emphasized the importance of thorough working of the butter to prevent the mottled appearance of the butter.

#### OUR EXPERIMENTS

*Amount of buttermilk and curd in butter and temperature of wash water are not cause of mottles.* The application of the conclusions of Van Slyke and Hart, in commercial buttermaking had long failed to avoid the appearance of mottles in butter. But, in order to demonstrate by exact experimental work the influence, or absence of influence, of the amount of curd in butter, and of the temperature of the washwater, as related to mottles, 36 separate churnings were made. This experiment was conducted in sets of four churnings each, the four churnings of each set being made from different portions of the same cream. All churnings were stopped when the butter granules were very small so as to facilitate removal of buttermilk. Two churnings of each set were washed with four washings and until the wash water was perfectly clear. The temperature of the wash water ranged from 40° to 50° F. The remaining two churnings of each set were not washed at all. One of the washed and one of the unwashed churnings of each set was salted, the other two churnings of each set were worked without salting. All lots were worked in the same manner and to the same extent. The working was purposely not carried quite so far as is necessary to avoid mottles. The results were very striking.

None of the unsalted butter showed mottles, whether washed or not washed. They all had a very compact texture and opaque whitish color. All of the salted churnings were mottled, regardless of whether the butter had been washed or not. The curd content in the washed butter ranged from 0.32 to 0.68 per cent, and the curd content in the unwashed butter ranged from 0.75 to 1.44 per cent.



These results indicated that the amount of curd in butter has not the slightest effect on the uniformity of color, that the temperature of the wash water has nothing to do with mottles and that the presence and distribution of the salt and brine is the only factor determining mottles.

*Curd enhances stability and permanency of emulsion of water in butter.* A clear understanding of the reasons for and the development of the succeeding experiments demands an explanation of the physical structure of butter.

Cream is a permanent emulsion of fat-in-skimmilk; the fat is the dispersed phase and the skimmilk the continuous phase. The emulsifying agents are: first, the process of milk secretion placing the butterfat into the skimmilk in a very fine state of division; second, the surface tension of the butterfat in excess of that of the skimmilk, holding these minute particles of fat in the shape of spheres or globules, and causing each globule to be surrounded by a concentrated layer of nitrogenous matter of the skimmilk; and third, the viscosity of the skimmilk which is caused by the hydrophylic colloids casein and albumin and by the lactose or milk sugar. Cream is a true and permanent emulsion. This emulsion can be destroyed only by agencies that offset and overcome the force of surface tension as will result for instance from solidification of the fat globules by cooling the cream, followed by coalescence into butter granules brought about by the churning process as shown by Hunziker (4), or by agencies that greatly diminish the viscosity and water-holding properties of the nitrogenous constituents in the cream as may result for instance from heating while the cream is at rest causing the butterfat to "oil-off."

The temperature at which the churning process is completed most rapidly is that at which the fat in the globular state becomes solid and of such consistency that, when they come together by impact, they coalesce and merge. As the churning process progresses a point is reached where the fat globules have united to such an extent, and the butter granules have become so large in proportion to their surfaces, that the surface tension and other factors are no longer sufficient to sustain the emulsion of

fat in skimmilk. The skimmilk recedes and the butter granules separate out, the butter "breaks." At this point the emulsion changes from a fat-in-skimmilk emulsion to a skimmilk-in-fat emulsion. The skimmilk is now called butter milk, and the butter milk is now the dispersed phase and the butterfat is the dispersing or continuous phase.

In properly washed butter the butter milk is largely replaced by water and therefore is removed from further consideration. The factors which now make butter a true emulsion and which establish the permanency of this emulsion are the temperature that keeps the fat in a solidified state and the nitrogenous matter or curd.

While the curd content of butter is small, averaging around 0.7 per cent, it plays an important rôle on the physical condition of the butter. During the churning process the curd distributes the water in the butter in a very fine state of division and it assists in sustaining this fine division. To be sure, pure water can, by mechanical means, be finely distributed throughout butterfat and by cooling it can be held there; but this is not readily possible by the churning process and without the presence of an emulsifying agent such as curd.

*Fine division of water droplets in butter produces opacity and diminishes intensity of yellow color.* When two substances insoluble in one another, and having different refractive indices are finely emulsified together, they form an opaque material of a lighter color. This opacity is caused by the bending of the rays of light at many and varied angles, due to the larger number of refracting and deflecting surfaces, and the degree of opaqueness is proportionate to the number and size of angles or refracting surfaces, i.e., due to the fineness of division. The same is true of one and the same substance reduced to a very fine state of division. Copper sulphate illustrates this phenomenon well. The large copper sulphate crystals present a deep blue color and are transparent. As the size of these crystals is reduced and the number of refracting surfaces increased, the transparency and the intensity of the blue color diminishes until the powder form is reached, which is completely opaque and almost white in color.

The emulsion in butterfat or butter, of water in a finely divided state causes such butter to be more opaque and lighter in color than the pure butterfat. Hence butter, which represents an emulsion of minute water droplets in butterfat, is less translucent, more opaque and lighter in color than butterfat.

Unsalted butter has a lighter and more opaque color than salted butter because in unsalted butter the water droplets are present in the original, finely divided state of the water-in-fat emulsion, while in salted butter this emulsion has been somewhat disturbed and partly destroyed by the action of the salt.

Furthermore, the greater the difference in the refractive index between the liquids or solids which are emulsified, the more abruptly are the rays of light bent and the more opaque becomes the emulsion. The difference between the refractive index of butter and of water is greater than between the refractive index of butter-fat and brine. This in turn increases the opacity of unsalted butter. In the case of salted butter the refractive index of the brine is more nearly the same as the refractive index of the butterfat. This enables the salted butter to be more nearly as translucent and as deep in color as pure butterfat.

Finally, any substance or condition that is capable of acting on and reducing the water-holding power of the emulsifying agent in butter—the curd—has a tendency to make the emulsification more difficult and less stable, causing the water droplets to be larger in size and fewer in number. Van Slyke and Hart have demonstrated that salt has a very decided action upon curd, as related to its water-holding property. Then again, the salt owing to its affinity for water, draws the water droplets together into larger aggregates, which further assists in the increase in size and decrease in number of water droplets.

That the size of the liquid droplets has a marked effect on the opaqueness of butter was effectively shown in the following manner:

Clear, pure, filtered butterfat was divided into four equal portions. To portion II was added 15 per cent, by volume, of distilled water; to portion III was added 15 per cent, by volume, of saturated brine; to portion IV was added 15 per cent, by

volume, of a viscous corn sugar syrup; portion I was kept pure for a check.

The four portions were vigorously shaken and simultaneously cooled. This was done in quart glass jars locked into a box holding the four jars and shaking back and forth on a mechanically operated eccentric. When this shaking and cooling had reduced the contents of the jars to a mushy consistency, the contents were removed from the jars, molded into bricks and set in the cooler for a day.

The four bricks were examined the next day for intensity and uniformity of color, and bricks II, III and IV were examined microscopically for size and counts of liquid droplets. The results of the microscopic examinations are tabulated below:

*Size of droplets*

No. II butterfat with water. . .	4 to 20 microns, average	6 microns
No. III butterfat with brine . .	4 to 20 microns, average	6-8 microns
No. IV butterfat with syrup. . .	20 to 50 microns, average	40 microns

The above figures show that prints II and III, representing the butterfat emulsified with water and with brine, respectively, contained droplets averaging 6 microns in diameter while print IV, representing the butterfat emulsified with syrup contained droplets which averaged 40 microns in diameter.

Prints II and III, representing the water and brine emulsions respectively, were alike in color and opaqueness. They were lighter and more opaque than print I representing the pure butterfat. Print IV, representing the syrup emulsion was plainly deeper yellow and clearer than prints II and III and it had very nearly the same appearance as print I.

The contrast was somewhat minimized by the copious incorporation of air in all four lots and yet the difference was quite marked. These findings demonstrated anew that the larger droplets in the case of the syrup emulsion, were capable of permitting the rays of light to enter sufficiently to give the print a deep, translucent color similar to that of pure butterfat, while the smaller droplets in the case of the water and brine emulsions deflected and refracted the rays of light sufficiently to lend to the prints an opaque whitish color.

*Decrease of water droplets in butter by evaporation gives butter a clearer and deeper yellow color.* The water-in-fat emulsion, representing butter, may be broken also and the opacity changed to translucency and a deeper yellow color, by evaporation of the moisture of the butter. This was strikingly demonstrated by the authors. A print (1 pound) of unsalted butter was set at room temperature (about 75° F.) for one month. At the end of this time the print was cut for examination. The cross section showed a sharply defined layer of a deep yellow and trans-

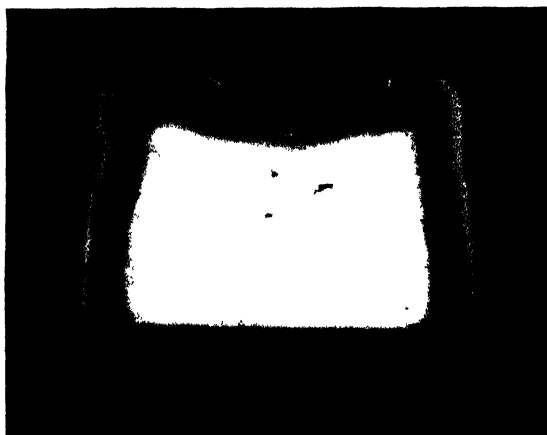


FIG. 1. CROSS SECTION OF PRINT OF UNSALTED BUTTER STORED AT ROOM TEMPERATURE FOR ONE MONTH

lucent color, while the interior of the same print had retained the opaque, light, straw color of normal unsalted butter. The deep yellow surface layer was about  $\frac{1}{4}$  inch thick. Figure 1 shows a photographic view of a cross section of this print. Analyses for moisture content are shown in table 1.

The above figures show that the moisture content in the outer layer, that part of the print that had changed to a deep yellow color and striking translucent appearance, had lost most of its moisture, while the interior of the print, that part which remained unchanged and had retained its original light straw

color, and opacity, contained from about six to thirteen times as much moisture. A similar effect was also produced in the cold by keeping unsalted butter in a desiccator containing sulphuric acid. By evacuating the desiccator containing sulphuric acid the time required to produce the same results was materially shortened.

These results simply show that by evaporation a part of the water-in-fat emulsion was broken and that the number of the small water droplets decreased very greatly. This in turn diminished the number of surfaces deflecting and refracting the rays of light. The light was capable of penetrating this outer layer and thereby to show more nearly the natural color and transparency of the butterfat.

TABLE 1  
*Per cent water in outer and inner portions of butter*

SAMPLE NUMBER	PER CENT MOISTURE	
	Outside layer deep yellow, translucent	Interior of print, straw color, opaque
1	1 77	9 11
2	1.25	17.00

The same experiment was made also with prints of salted butter but no surface layer of deeper yellow and translucent color appeared. The butter of the entire print in each case remained uniform in color and retained its original color. The reason for this lies in the fact that the salt has already broken down the water-holding power of the curd and diminished the number and increased the size of the water droplets to the extent of giving the butter a deeper yellow and more translucent color, and as the water evaporates on the surface, the concentrated brine and salt crystals on the surface cause more water from the interior to migrate toward the surface to take its place. Hence the whole print remains quite uniform in composition and in color.

*Action of salt causes migration of water and brine in butter.* That there is such a migration of water in salted butter, and that a similar migration of water is not found in unsalted butter

is conclusively demonstrated by the results of the following experiment and shown by the accompanying photograph, figure 2.

One print each, of salted butter and unsalted butter was submerged in a water solution of acid fuchsin. After several days the prints were removed and cut in two. The body of the salted butter showed a red coloration throughout, while the body of the unsalted butter had retained its original, pure yellow color.



FIG. 2

UNSALTED BUTTER

SALTED BUTTER

Both prints were immersed in a watery solution of fuchsin. The salted butter became permeated with a net-work of red coloration while the unsalted butter retained its original color. This illustration represents cross sections of the two prints through the center.

The fact that the salted butter has a less compact body, and is, therefore, more prone to be leaky than unsalted butter, is well known. The accompanying photograph, figure 3, shows a typical microscopical fissure, as found in salted butter. These fissures are caused by the action of the salt on the curd, causing the emulsion to break, the fine droplets of water to run together and to fill the spaces between the butter granules with larger aggregates of liquid. Such fissures are not present in unsalted butter and unsalted butter does not become leaky.

*Salt disturbs water-in-fat emulsion and thereby causes mottles.* It has been shown that salt causes the emulsion of water-in-fat to be less stable, due to its action on the casein, the emulsifying agent, and due to the great affinity of the salt for water. It remains to be shown that it is this same action of the salt,



FIG. 3. SHOWING MICROSCOPIC FISSURES OF BUTTER UNDER LOW MAGNIFICATION.  
MAGNIFIED 15 TIMES

that is the cause of mottles. For this purpose the following experiment was conducted:

1. Slabs of salted butter which was perfectly uniform in color, and slabs of unsalted butter, were placed in brine containing 25 per cent salt, and held there over night.
2. Slabs of the same salted and unsalted butter were placed in water, and held there over night.



Examination of the butter the following morning showed the following results:

Salted butter	{	In brine—color unchanged, no mottles
	{	In water—profusely mottled with lighter colored dapples
Unsalted butter	{	In water—color unchanged, no mottles
	{	In brine—profusely mottled with darker colored blotches

Six sets of the above tests were made and the results were identical and very striking. The reason for these results is obvious. When salted butter is immersed in water we have two solutions of different concentrations, i.e., the concentrated salt solution—the brine—in the butter, and the very dilute solution—the water—surrounding the butter. The brine, following the physico-chemical law of diffusion of a solute, will migrate in part from the butter into the water surrounding the butter, where it will tend to make both solutions of equal concentration. And the water surrounding the butter will, by osmosis and otherwise, migrate to the droplets in the butter of greater concentration, causing them to enlarge and become more dilute. This migration and interchange of liquids and this change in concentration causes the less firmly held droplets of brine to run together, simultaneously exposing the portions of butter in which the more firmly held minute droplets are localized. And these portions, being opaque and of whitish color cause the appearance of light-colored, opaque dapples in the butter.

In the case of the unsalted butter immersed in brine, again there are two solutions of different concentrations, the water in the butter and the brine surrounding the butter. Hence here too, because of their difference in concentration, there is migration and interchange of brine and water, and in this case the result is the reverse of that of the salted butter in water. The brine acts upon the emulsion, causing a partial breaking down of the latter. It permits the more loosely held droplets of water in the butter to run together and to form large droplets of

brine. These larger droplets are formed at the expense of the smaller droplets and the thus formed localized sections of larger droplets give these portions a clearer and deeper yellow color and cause the appearance of the darker blotches, making the butter mottled.

In the case of the salted butter immersed in brine the concentration of the two liquids—the brine in the butter and the brine surrounding the butter, is so near alike, that the tendency of the two liquids to migrate is practically removed; there is no cause for interchange of liquids. The droplets of brine in the butter remain where the working process has placed them, there is no change of color, and the butter does not become mottled.

In the case of the unsalted butter immersed in water the situation is very similar as with salted butter in brine. There is only one liquid; in this case it is the water, water in the butter and water surrounding it. Hence there is no cause for interchange of liquids. The droplets of water remain where the working process has placed them, there is no change of color, and the butter does not become mottled.

*Proper working of salted butter restores the water-in-fat emulsion sufficiently to prevent mottles.* Having established the fact that salt when added to butter partly breaks the emulsion of water-in-fat, by its action on the curd and its affinity for water, causing an uneven distribution of large and small water droplets in the butter, and having demonstrated that small water droplets produce an opaque and light color, while large water droplets produce a clearer and deeper yellow color in butter, the next step was to determine the effect of working the butter, on the size, number, and distribution of the water and brine droplets. Or in other words, to what extent is the working process capable of restoring the water-in-fat emulsion that was disturbed by the action of the salt, and to thereby avoid the appearance of mottles in commercial butter. For when butter is uniformly and sufficiently worked mottles do not occur.

For this purpose four churnings were made, using cream from the same vat for each churning. The amount, condition, and preparation of the cream is tabulated in table 2.

The churn used was a Disbrow D-2 Dairy Junior with solid glass end. It has one worker roll, working against an idler roll, which is located between shelf and worker. In this churn the butter is worked once only with each revolution of the churn. This churn has a listed capacity of 370 pounds of cream and 110 pounds of butter. Two hundred pounds of cream were used for each churning, but even this amount overloaded the worker

TABLE 2

*Churning record*

Total pounds of cream in vat 2050 pounds

	CHURNINGS			
	No. 1	No. 2	No. 3	No. 4
Pounds of cream per churning .....	200	200	200	200
Per cent acid before neutralization. ....	0.65	0.65	0.65	0.65
Per cent acid before starter added ....	0.235	0.235	0.235	0.235
Per cent acid at churn.....	0.288	0.270	0.279	0.290
Pounds starter added.....	30	30	30	30
Per cent fat in cream at time of churning	33 25	33 25	33.25	33.25
Temperature of pasteurization for thirty minutes, ° F.....	145	145	145	145
Cream cooled in vats to, ° F.....	40	40	40	40
Held at 40° F, hours.....	5	5	5	5
Churning temperature, ° F.....	50	49	50	50
Held at churning temperature, hours ...	3½	7	10½	14
Minutes churned.....	50	77	57	62
Temperature of buttermilk, ° F.....	56	54	55	56
Temperature of wash water, ° F.....	50	51	50	50
Pounds salt added.....	3 3	None	3.3	None
Worked.....	With drain open		With drain closed	

somewhat, so that it required a few revolutions before the butter all went through the workers.

The churnings were all stopped when the granules had reached the size of wheat grains. The butter was washed twice with about 160 pounds of water at a temperature a few degrees lower than that of the buttermilk (see table 2).

Samples of the butter were taken at different stages of the working process—after working 6, 12, 18, 26, 34, 42, 66 and 120 revolutions, for chemical analysis, for microscopical examinations,

and two prints were taken for future inspection of body and color. The results of these analysis, examinations and inspections are tabulated in tables 3 and 4.

Table 4 shows that under the conditions of operation, with properly chilled cream, amount of butter and type of water used, it required 34 revolutions of working before the salted butter stopped becoming mottled. (However in this small churn, smaller amounts of salted butter had been made which showed no mottles at all when worked only 28 revolutions.)

No mottles appeared in the unsalted butter at any stage of the working process. This corresponds with the common experience. Unsalted butter does not show mottles because, the emulsion of the fine water droplets is not disturbed, it remains intact. There is no salt or brine or other agency present which causes a migration due to difference in concentration or has any effect upon the emulsion after the completion of the working process and while the butter is at rest.

No mottles appeared in the salted butter at the churn. This too is in accordance with common experience. The reason for this lies in the fact that, even in incompletely worked butter the large droplets of brine are sufficiently uniformly distributed to cover up the small and finely divided droplets of water and to give the entire body a uniformly deep yellow and clear appearance.

Mottles appeared in all incompletely worked, salted butter in two days, because in this butter the fusion of brine and water was incomplete. Therefore, a diffusion took place of the salt from the more concentrated droplets to the less concentrated droplets. Also the water, by osmosis, enlarged the more concentrated droplets until the salt solution throughout the butter became of the same concentration. This diffusion of the brine and movement of the water by osmosis partially broke down the emulsion in the butter at rest, causing localized aggregations of very minute and well emulsified droplets of water to be exposed and these portions showed themselves as opaque, whitish dapples.

TABLE 3  
*Color and body of butter at different stages of working process*

REVOLUT- IONS	AGE AT TIME OF EXAMINATION	CHURNING I—SALTED DRAIN OPEN	CHURNING II—UNSALTED DRAIN OPEN	CHURNING III—SALTED DRAIN CLOSED	CHURNING IV—UNSALTED DRAIN CLOSED
6	At churn	No mottles, golden*	No mottles, straw†	No mottles, golden	No mottles, straw
	2 days	Mottled, golden	No mottles, straw	Mottled, golden	No mottles, straw
	1 week	Mottled, golden	No mottles, straw	Mottled, golden	No mottles, straw
12		Loose grain	Compact	Loose grain	Compact
	At churn	No mottles, golden	No mottles, straw	No mottles, golden	No mottles, straw
	2 days	Mottled, golden	No mottles, straw	Mottled, golden	No mottles, straw
18	1 week	Mottled, golden	No mottles, straw	Mottled, golden	No mottles, straw
		Good body	Good body	Good body, slightly leaky	Good body
	At churn	No mottles, golden	No mottles, straw	No mottles, golden	No mottles, straw
26	2 days	Mottled, golden	No mottles, straw	Mottled, golden	No mottles, straw
	1 week	Slightly mottled, golden	No mottles, straw	Mottled, golden	No mottles, straw
		Slightly mottled, golden	No mottles, straw	Slightly mottled, slightly lighter	No mottles, straw
		Good body	Good body	Good body, slightly leaky	Good body

34	At churn	No mottles, slightly lighter	No mottles, straw	No mottles, slightly lighter	No mottles, straw
	2 days	No mottles, slightly lighter	No mottles, straw	No mottles, slightly lighter	No mottles, straw
	1 week	No mottles, slightly lighter	No mottles, straw	No mottles, slightly lighter	No mottles, straw
		Good body	Good body, slightly short grain	Good body, slightly leaky	Good body
42	At churn	No mottles, lighter	No mottles, straw	No mottles, lighter	No mottles, straw
	2 days	No mottles, lighter	No mottles, straw	No mottles, lighter	No mottles, straw
	1 week	No mottles, lighter	No mottles, straw	No mottles, lighter	No mottles, straw
		Good body, slightly overworked	Good body, short grain and slightly salty	Good body, slightly leaky	Good body
66	At churn	No mottles, much lighter	No mottles, straw	No mottles, much lighter	No mottles, straw
	2 days	No mottles, much lighter	No mottles, straw	No mottles, much lighter	No mottles, straw
	1 week	No mottles, much lighter	No mottles, straw	No mottles, much lighter	No mottles, straw
		Body overworked, slightly sticky, dense	Slightly salty	Good body, slightly leaky	Slightly salty
120	At churn	Working discontinued	Working discontinued	No mottles, straw	No mottles, straw
	2 days			No mottles, straw	No mottles, straw
	1 week			No mottles, straw	No mottles, straw
				Fair body, slightly salty	Fair body, slightly salty

\* Golden—refers to golden yellow color.

† Straw—refers to light straw color.

As the working process continued, the fusion of brine and water became more and more complete and their re-emulsification more and more thorough, until, at 34 revolutions, it was complete enough to prevent any further diffusion of salt, or migration and interchange of liquids in the butter at rest, and therefore made impossible the appearance of mottles. This condition is reached when the butter has been worked sufficiently to give it a tough, waxy and plastic texture.

The entire series of samples of each churning showed a gradual gradation of color, the color becoming lighter and duller as the

TABLE 4

*Per cent salt and moisture in butter at different stages of working process*

REVOLUTIONS WORKED	CHURNING I—SALTED, DRAINED		CHURNING II— NOT SALTED, DRAINED	CHURNING III—SALTED, NOT DRAINED		CHURNING IV—NOT SALTED, NOT DRAINED
	Salt	Moisture	Moisture	Salt	Moisture	Moisture
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
None	0.00	21.76	20.84	0.00	22.16	19.54
6	1.10	14.14	15.38	1.17	14.25	14.77
12	1.11	14.54	14.92	1.17	14.44	15.04
18	1.24	14.97	15.42	1.19	14.92	15.83
26	1.16	14.94	15.97	1.45	15.73	16.44
34	1.20	15.06	16.06	1.52	16.45	16.91
42	1.18	15.20	16.24	1.62	17.09	18.40
66	1.26	15.43	16.36	1.86	18.96	19.24
120				2.38	22.78	21.42

working process progressed. This was especially pronounced in the case of the salted butter, which at six revolutions had a bright, live, deep yellow and translucent color, while the unsalted butter had a straw color. The deep yellow color of the salted butter diminished with the continuation of the working process, and at 120 revolutions, the salted butter was practically as opaque and had as light a straw color as the unsalted butter at the beginning of the working process. This phenomenon is obviously due to the fact that at 120 revolutions the salted butter had been reduced to as fine an emulsion of water droplets in fat, as prevailed in the unsalted butter at the beginning of

the working process. And this fine emulsion with its multitude of minute brine droplets, bending the rays of light, made the butter look opaque and of a straw color. For corroboration of these facts see also the microscopic examination of the butter.

It is interesting to note also in table 4, showing the per cent moisture at the different stages, that before working and salting, the butter contained around 20 per cent moisture. During the first six revolutions of working the bulk of the loose water had escaped and from then on there was a gradual reincorporation of this moisture until, at 120 revolutions, practically all the previously expelled water had been reincorporated in the butter. The decrease in moisture content during the first six revolutions of working was greater in the case of the salted butter than in the unsalted butter, because of the action of the salt on curd and moisture. These results fully corroborate the findings of Hunziker, Mills and Spitzer (5) and they demonstrate anew that moisture control is not so much a matter of incorporating extraneous water, as it is a problem of regulating the escape of the moisture that is originally present in the butter.

*Microscopic examination of butter.* The most striking proof of the correctness of the preceding deductions concerning the relation of the size and distribution of the water droplets in butter to mottles is represented in the results of the microscopic examinations of the butter from beginning to end of the working process, as shown in the accompanying photographs, figures 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 and 17.

These examinations were made in accordance with the following procedure: A small "dab" of butter was placed on a slide and a cover-glass was laid on this butter. With another slide the cover-glass was carefully pressed down. This made a thin film of butter between the first slide and the cover-glass. The darkfield illuminator was used, both for examination and counting and for taking photographs. It was fully realized that any count of the water droplets without a measurement of the thickness of the film can only be an approximation. But by counting only those water droplets that are in focus and appear as water droplets, the counts are necessarily limited to the water drop-



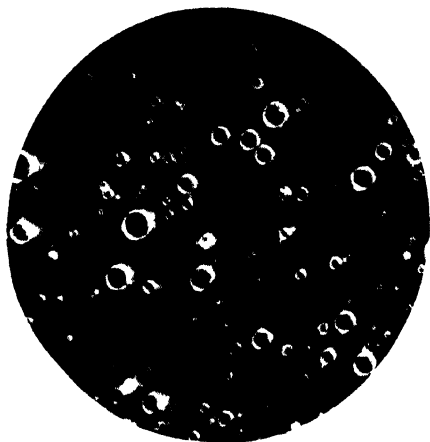


FIG. 4. Worked 6 revolutions.  
Mottled

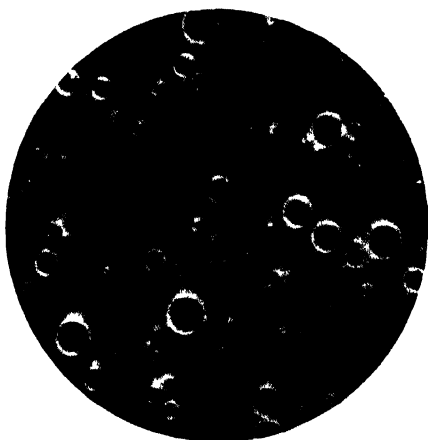


FIG. 5. Worked 12 revolutions.  
Mottled

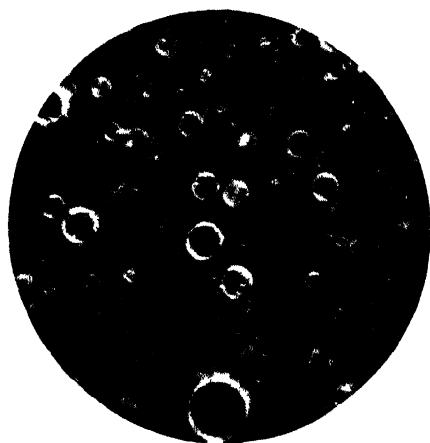


FIG. 6. Worked 18 revolutions.  
Mottled

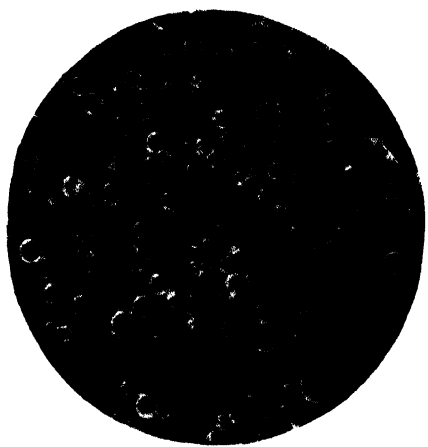


FIG. 7. Worked 26 revolutions.  
Slightly mottled

MICROSCOPIC VIEW OF WATER DROPLETS IN *Salted BUTTER* AT DIFFERENT STAGES OF WORKING PROCESS. MAGNIFIED 740 TIMES



FIG. 8. Worked 34 revolutions.  
Not mottled



FIG. 9. Worked 42 revolutions.  
Not mottled

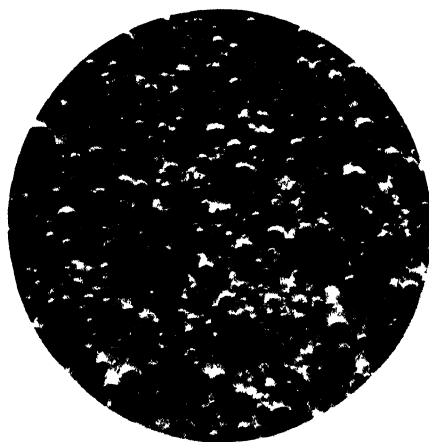


FIG. 10. Worked 66 revolutions. Not mottled

MICROSCOPIC VIEW OF WATER DROPLETS IN *Salted* BUTTER AT DIFFERENT STAGES  
OF WORKING PROCESS. MAGNIFIED 740 TIMES

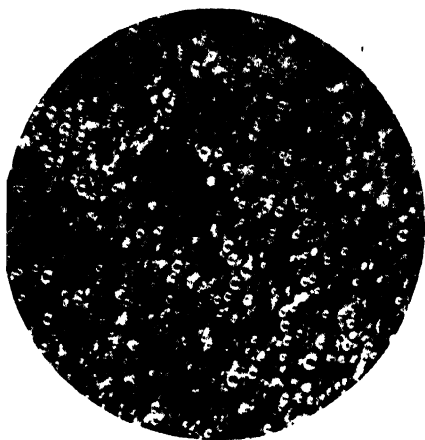


FIG. 11. Worked 6 revolutions.  
Not mottled

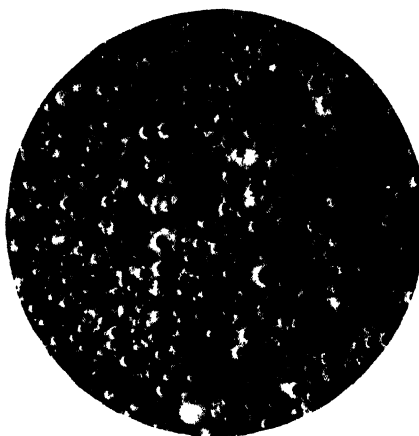


FIG. 12. Worked 12 revolutions.  
Not mottled

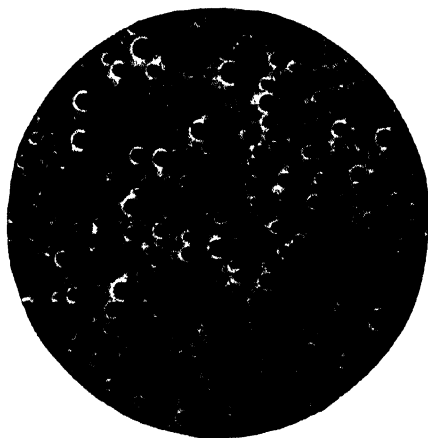


FIG. 13. Worked 18 revolutions.  
Not mottled.

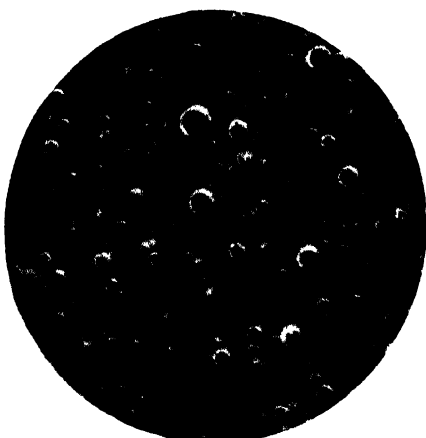


FIG. 14. Worked 26 revolutions.  
Not mottled

MICROSCOPIC VIEW OF WATER DROPLETS IN *Unsalted* BUTTER AT DIFFERENT STAGES OF WORKING PROCESS. MAGNIFIED 740 TIMES



FIG. 15. Worked 34 revolutions.  
Not mottled

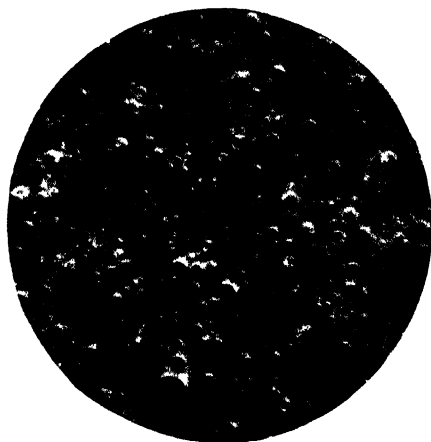


FIG. 16. Worked 42 revolutions.  
Not mottled

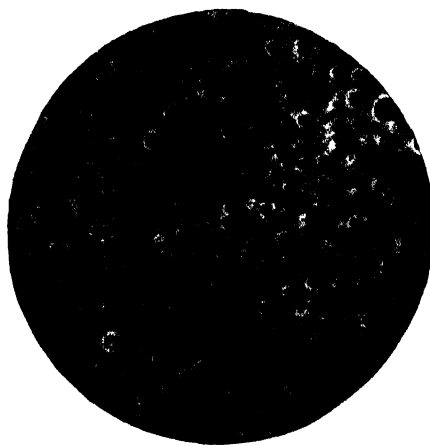


FIG. 17. Worked 66 revolutions. Not mottled

MICROSCOPIC VIEW OF WATER DROPLETS IN *Unsalted* BUTTER AT DIFFERENT STAGES OF WORKING PROCESS. MAGNIFIED 740 TIMES



FIG. 18. Whitish, opaque dapples

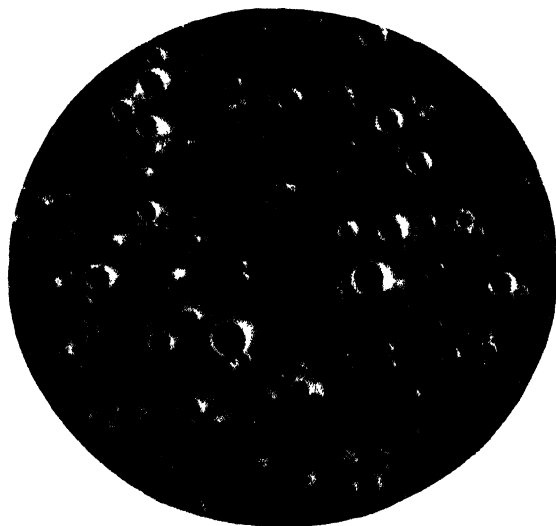


FIG. 19. Deep yellow, translucent blotches

MICROSCOPIC VIEW OF WATER DROPLETS IN LIGHT AND DARK DAPPLES FROM THE  
SAME PRINT OF MOTTLED BUTTER. MAGNIFIED 740 TIMES

lets situated in approximately the same depth of the film, thereby making possible comparable counts. This method proved successful in securing uniformity of results of duplicate examinations of the same sample and it is by this method that the comparative counts of the water droplets per field were made on all samples of butter.

TABLE 5

*Counts of water droplets in microscopic field and moisture content of salted and unsalted butter at different stages of the working process*

NUMBER OF REVOLUTIONS WORKED	CHURNING I—SALTED, DRAINED			CHURNING II—UNSALTED, DRAINED		
	Water droplets		Water	Water droplets		Water
	Total number	Diameter above 4 microns		Total number	Diameter above 4 microns	
			<i>per cent</i>			<i>per cent</i>
	6	357	8	14 14	820	0
12	232	17	14 54	700	0	14 92
18	465	12	14 97	750	2	15 42
26	455	14	14 94	740	5	15 97
34	542	8	15 06	860	0	16 06
42	572	4	15 20	897	1	16 24
66	552	9	15 43	970	3	16 36
	CHURNING III—SALTED, NOT DRAINED			CHURNING IV—UNSALTED, NOT DRAINED		
6	542	8	14 25	735	4	14 77
12	450	5	11 44	805	9	15 04
18	602	6	11 02	795	5	15 83
26	445	16	15 73	650	7	16 44
34	490	11	16 45	915	0	16 90
42	635	8	17 09	1162	0	18 40
66	785	8	18 96	1090	0	19 24
120	977	11	22 78	1025	6	21 42

*Salted butter has fewer and larger water droplets than unsalted butter.* Further comparative microscopic examinations were made of miscellaneous salted and unsalted butter. In these examinations three groups of sizes of water droplets of each field were recorded, namely, (1) all visible water droplets below two microns in diameter, (2) all water above two and below 5 microns in diameter, and (3) all water droplets above 5 microns that are still in globular form. The results are shown in table 6.

The above results bear out the findings of the previous experiment. The water droplets in the unsalted butter are much more numerous than those in the salted butter and there is a greater predomination in the number of small droplets in the case of the unsalted butter, while in the salted butter there are more large droplets than in the unsalted butter.

TABLE 6

*Number and size of water droplets in salted and unsalted butter*

SIZE OF WATER DROPLETS	NUMBER OF WATER DROPLETS					
	Salted butter			Unsalted butter		
	Sample I	Sample II	Average	Sample III	Sample IV	Average
Diameter less than 2 microns* . . . . .	430	470	450	1050	1270	1160
Diameter between 2 and 5 microns. . . . .	72	54	63	94	66	80
Diameter above 5 microns† . . . . .	6	7	7.5	1	0	0.5

\* The smallest represents the smallest visible under a magnification of 740 diameters.

† The largest droplet still in globular form was 16 microns

TABLE 7

*Number and size of water droplets in deep yellow blotches and in opaque whitish dapples of mottled butter*

SIZE OF WATER DROPLETS	NUMBER OF WATER DROPLETS	
	Deep yellow blotches	Light dapples
Diameter less than 2 microns . . . . .	493	1600
Diameter between 2 and 5 microns . . . . .	80	56*
Diameter above 5 microns . . . . .	10	0

\* Very few above 3.5 microns.

*White dapples of mottled butter contained multitude of minute water droplets and deep yellow blotches of mottled butter contained fewer and larger water droplets.* \* Next, microscopic examinations were made of the light dapples and deep yellow blotches of mottled butter taken from the same package of butter, with the results shown in table 7.

The above figures and the accompanying illustrations, figures 18 and 19, furnish most convincing additional proof of the fact already established by the foregoing results, that small water droplets give the butter an opaque, whitish appearance, while large water droplets or the absence of water droplets permit the butter to appear more nearly in the natural deep yellow and clear color of butterfat. And that, therefore, an uneven distribution of small and large water droplets causes butter to be mottled, the light-colored dapples appearing in those portions that harbor a multitude of exclusively very minute water droplets while the deep-yellow blotches represent portions in which there are fewer and larger water droplets.

#### SUMMARY

1. Mottles appear only in salted butter.
2. Mottles appear in salted butter in which the working has been incomplete or lacking in uniformity.
3. Large numbers of very minute water droplets cause butter to be opaque and of light color.
4. The fewer and the larger the water droplets, the deeper yellow and clearer the color of the butter.
5. Salt disturbs the emulsion of water-in-fat in butter, causing a reduction in number and increase in the size of the water droplets, and giving such butter a deeper yellow color than it had before salting.
6. Mottles do not appear at the churn because, even in incompletely worked, salted butter, there is a sufficient distribution of the larger droplets to hide the localized units of the small droplets.
7. In incompletely or unevenly worked, salted butter, mottles appear about six to twelve hours after working.
8. The late appearance of mottles in butter that is destined to become mottled, is due to the fact that in such butter the working process did not accomplish a complete fusion and re-emulsion of the water and brine. When this butter is set at rest, an equalization or interchange of the brine and water sets in, owing to the difference in concentration between the different droplets. The



water, by osmosis, migrates from the droplets of low concentration to those of greater concentration and vice versa, causing the droplets to become larger. This action results in a partial breaking down of the emulsion, liberating and effecting a running together of the less firmly held droplets and drops. This in turn uncovers and exposes to view sections containing a multitude of minute water droplets, which result in the appearance of opaque, whitish dapples on the one hand; and there is a deepening of the color in those portions where, because of this running-together of water droplets, there are fewer and larger droplets. And the butter looks mottled.

9. The proper working of butter brings about the necessary fusion of the water and brine and their re-emulsification removing the cause of mottles.

10. In order to prevent mottles, butter must be worked sufficiently to accomplish this fusion and re-emulsification of water and brine. This point is usually reached, when the butter has been reduced, by working, to a plastic, tough and waxy body. The working must be uniform throughout the churn; overloaded workers and workers improperly set, loose or slipping, will not work butter evenly and are prone to produce mottled and wavy butter.

#### REFERENCES

- (1) STORCH: Untersuchung ueber einige häufig vorkommende Konsistenz fehler und die Ursachen ihres Entstehens, sowie ueber den Bau der Milch kugélchen, Milchzeitung, 1897.
- (2) VAN SLYKE AND HART: New York (Geneva) Agr. Expt. Sta. Bulletin 1905.
- (3) SAMMIS AND LEE: Mottles in butter. 28th and 29th Annual Report, Wisconsin Agr. Expt. Sta. 1911 and 1912.
- (4) HUNZIKER: The Butter Industry. First edition, 1920.
- (5) HUNZIKER, MILLS AND SPITZER: Moisture control of butter. Purdue Bulletin 160, 1912.

## SUGGESTIONS REGARDING THE CONTROL OF MUNICIPAL MILK SUPPLIES<sup>1</sup>

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In an earlier number of this JOURNAL there appeared a report of the American Dairy Science Committee on Milk Quality (1) in which it was pointed out that everything really essential in milk quality was included under food value, healthfulness, cleanliness and keeping quality.

Since the appearance of that report questions have arisen as to how far this view of milk quality may be related to the routine procedures in municipal milk control.

So many suggestions have been received through friendly discussions of this subject that it is practically impossible to present a paper without unwittingly appropriating the ideas of others while at the same time it might be embarrassing to attribute any formal expression to any particular friend who might in turn fail to recognize it or be loath to approve it exactly in the form it might be stated. Accordingly the situation will be best met if the reader will kindly give credit for anything which may seem good to friendly advisers while holding the authors responsible for anything with which he does not find himself in agreement.

There are many and complex problems even in the field of milk control. These arise partly because of the nature of milk problems and partly because of the varying natural and developmental conditions which exist in the wide stretch of territory included within our country. In the northern portions the prevailing temperatures are arctic and ice is almost too abundant while in the southern portions tropical temperatures rule and natural ice is unknown. Not only should these extreme con-

<sup>1</sup>NOTE.—After this manuscript was submitted there appeared N. Y. Agr. Exp. Sta. Technical Bul. 71. A method for the preliminary detection of abnormal milks. This suggests the use of brom-cresol purple as an indicator.

ditions be considered but it is well to remember that the larger part of our most quoted milk regulations have developed in a series of cities lying in a narrow zone where natural ice in winter is the normal experience and the summer temperatures while high for a time do not unduly increase the temperature of the water available for cooling the milk.

The developmental conditions are as varied as the climatic ones. In some of our cities efficient, constructive work with milk supplies has been in progress for more than a generation (2) exerting an influence upon both the conditions under which milk is produced and handled and upon the public appreciation of good milk. On the other hand in many of the other cities little or nothing in the way of milk control has been seriously undertaken. Moreover the quality of the milk supply of the rural third of our population has received little consideration. When careful study is given to this latter phase of the question it will undoubtedly be found that like the water supply the rural milk supply taken as a whole is not equal in quality to that of the larger cities and is scarcely comparable with that of the smaller ones.

In view of the fact that the milk supplies of at least three-fourths of our cities are at present imperfectly controlled it seems best to confine these suggestions to the more universal problems of milk control. It is frequently noted that a municipality in beginning the improvement of its milk supply often copies the regulations which have been developed in another city as the result of a long evolution. Such attempts to quickly attain perfection are rarely successful. There are certain qualities to be striven for in every milk supply. In any given region the order and method of attaining them will have much in common. The detailed steps by which they may be attained in any community depends upon the local conditions and personalities involved (3).

Official oversight of anything which comes into intimate touch with all the people can not proceed far in advance of public sentiment. Accordingly attention must be given to the public mind regarding milk and the possibility of so directing milk improve-

ment as to increase intelligent interest in the subject. Progress is the logical result of the best elements in the situation working together. The tendency of the press to announce milk crusades, suspicion on the part of the milk men that the health officials are not fair minded and the feeling on the part of the consumers that the milk is hopelessly bad are usually all to be met and overcome.

If the more intelligent members of the community are asked to express in non-technical language their ideas regarding the characteristics of a good milk supply they will insist that it be such that they can use it without danger of disease, that it be fairly rich in cream, that it carry nothing offensive to the aesthetic sense and that it remain sweet for a reasonable time after delivery. In short the consuming public desires a milk which is safe, rich, clean and sweet. Any system of control which they understand is striving to provide them with such an article tends to enlist their support.

Most workers in milk improvement will insist that these are precisely the objects which they have had constantly in view. However, so long as their private and public statements are directed primarily toward minimum legal limits of composition, barn scores and bacterial counts, it is a fair question as to how much the public is to be blamed for not appreciating the connection between such things and the characteristics of a milk supply in which the public is interested.

Hoping thereby to make more evident the relation between the kind of milk which the public desires and the regulations which will assist in its production, milk regulations will be discussed under the headings of milk which is safe, rich, clean and sweet.

#### SAFE MILK

The safety of the milk supply can be guaranteed either by a supervision of the health of the cows and people concerned in its production or by treatment of the milk itself.

There is a natural and widespread feeling that it is better to protect milk from infection than to free it from infection. Ex-

perience with "certified" milk production has shown that a careful, periodic examination of the cows and people supplemented by constant oversight will reduce the danger of contamination to low limits. On the other hand the expense incident to such a careful, frequent examination and oversight is so great that no large city has yet provided adequate funds for such supervision of its milk supply. When properly done such a supervision entails an economic burden of some cents per quart.

The other method of rendering the milk safe from the danger of carrying disease germs is positive pasteurization followed by protection from subsequent contamination.

Positive pasteurization (4) is the process by which every portion of the milk so treated is heated to a temperature of 140° to 145°F., held thereat for thirty minutes, and then cooled to a temperature of 50°F. or lower, the degree and time of heating, holding and cooling being invariably recorded by tested automatic device, the records from which are dated daily on removal from the device, and are checked at regular intervals by health authorities.

Protection from subsequent contamination (5) means filling the milk, immediately after pasteurization and at the place thereof, in reasonably sanitary surroundings (having special reference to pure water supply, complete and safe disposal of human excreta, and control of fly prevalence), by healthy handlers, into clean and sufficiently steamed containers (205°F. for two minutes), which, having been machine capped (in the case of bottles), are stored at a temperature lower than 50°F. until delivered within twenty-four hours to consumers.

Milk handled in accord with the above definitions of pasteurization and protection is justly entitled to be sold as Pasteurized milk.

Pasteurization and later positive pasteurization was first introduced by milk dealers as an adjunct to their business and have later been generally accepted by health authorities for health purposes.

The advantage of pasteurization lies in the fact that its close supervision is within the financial possibility of practically any

municipality and the efficiency of its protective action is correspondingly high. It has the advantage, particularly in a large milk business, of so reducing waste as to practically offset the pasteurizing expense, leaving little or no economic burden upon the milk. In the larger cities the requirement of positive pasteurization results in such manifest improvement of the milk supply at so small an expense that the milk industry can be counted upon to support the movement. In the small cities and in the rural communities, where the expense incident to pasteurization become a tax upon the business, opposition to pasteurization requirements becomes more pronounced.

An ideal protection of the milk would consist of a careful supervision of the health of the cattle and of the people concerned in its production and handling, coupled with a positive pasteurization and prevention of subsequent contamination. The least expensive, most easily attained, and most productive step in this direction is usually the requirement of positive pasteurization and protection from subsequent contamination. This should be accompanied by such further efforts at safeguarding the health of the cattle and of the people connected with the milk production as the circumstances will permit.

#### RICH MILK

The public purchases milk primarily as a food and quite universally judges the richness or food value of the milk by the amount of cream.

Because milk is peculiarly valuable as a source of materials for building and repair of tissue, and because it is the most readily available source of substances essential to the development of the young and the well being of the adult, any direct comparison between milk and other food substances on the basis merely of calories of available energy is distinctly unfair to milk. However the calorific content is a fair and convenient basis for comparing one normal milk with another.

A study of the composition of milk as shown by samples collected in the open market gives somewhat variable results because

many of the samples so taken are from milk whose composition has been altered. A compilation of the results of analysis of milk of known purity shows quite accordant results. Table 1, taken from Circular 235 of the Illinois Agricultural Experiment Station (6), shows the progressive increase in net calorific energy per quart in normal milks ranging from 3 to 7 per cent in fat content.

From table 1 it is seen that a normal 3 per cent milk furnishes slightly less than 550 calories while a 4 per cent milk furnishes slightly more than 660 calories per quart. This variation in fat content is well within the range ordinarily found upon our local markets. However, if the 3 per cent milk is retailing at 15 cents per quart, on the basis of food value the 4 per cent milk is then worth 18 cents per quart, or put in another way, if a workingman should adopt a strictly milk diet and get his 3000 calories per day solely from milk he must drink practically a quart extra per day in getting this amount of energy from 3 per cent milk.

As the fat content of our local milk supplies retailing at the same price, particularly in the smaller and medium sized cities, ranges from 3 per cent to 5.5 per cent, it is important that the consumer be advised of the importance of more carefully considering food value in buying milk. A requirement that bottled milk be labeled as to its fat content is probably the surest way of getting accurate information on this subject to the consumer. With this should go the requirement that the fat content of the milk should always be up to the statement on the bottle.

While these statements lay stress upon the desirability of rich milk when all is selling at the same price, the fact should not be overlooked that all safe, clean, sweet milk is a peculiarly valuable food. Practically one-half of the food value of a normal milk remains after the fat has been removed and an increase in the use of safe, clean skim milk should be encouraged provided it is sold with a clear understanding that it is skim milk.

TABLE 1  
*Energy values of milks*

PROTEIN	FAT	CARBOHY- DRATES	FOOD SUBSTANCE PER QUART*	CALORIES PER GRAM	CALORIES PER QUART	TOTAL CALORIES PER QUART
1	2	3	4	5	6	7
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>grams</i>	<i>calories</i>	<i>calories</i>	<i>calories</i>
2.648			25.87	4	103.48	
	3.00		29.31	9	263.79	
		4.596	44.90	4	179.60	546.87
3.068			29.96	4	119.84	
	3.498		34.18	9	307.62	
		4.903	47.90	4	191.60	619.06
3.044			29.74	4	118.96	
	3.994		39.02	9	351.18	
		4.875	47.63	4	190.52	660.66
3.082			30.11	4	120.44	
	4.516		44.12	9	397.08	
		4.958	48.44	4	193.76	711.28
3.62			35.37	4	141.48	
	5.048		49.32	9	443.88	
		4.922	48.09	4	192.36	777.72
3.743			36.57	4	145.28	
	5.534		54.07	9	486.63	
		4.93	48.17	4	192.68	825.59
3.992			39.00	4	156.00	
	5.94		58.03	9	522.27	
		4.878	47.66	4	190.64	868.91
4.12			40.25	4	161.00	
	6.50		63.52	9	571.68	
		4.90	47.87	4	191.48	924.16
4.22			41.23	4	164.92	
	7.00		68.39	9	615.51	
		4.84	47.29	4	189.16	969.59

\* In computing these values, 977 grams has been used as the weight of one quart of milk. This is the weight of a quart of milk of specific gravity 1.0323, or nearly the average specific gravity of milk. The use of the minimum or maximum limits of specific gravity of normal milk (1.029-1.035) would change the values so slightly as to be negligible in so far as the purpose of this circular is concerned.



## CLEAN MILK

The public uniformly demands and should have clean milk. There is, however, some difference of opinion as to what should be considered as dirt in milk. Some hold that it will simplify public consideration of milk problems and ordinances to consider the term dirt in milk as applying to such foreign matter as would ordinarily be covered by that term; while others hold that the bacteria, being in general objectionable, should also be considered as dirt.

Much can be said in favor of the latter conception but under it one is at once confronted by the fact that bacteria are always present even in the best of milk and in the hands of the consumer their numbers vastly increase. The consumer is slow to understand when told that all milk is dirty and that even under his most careful handling it rapidly becomes more dirty. The enthusiastic advocates of sour milk drinks also resent the implication that they are urging the use of dirty milk.

Administratively considered the means available for detecting the presence in milk of dirt, in the ordinary meaning of that term, and of bacteria are entirely different. Therefore, the inclusion of dirt and bacteria under one official heading makes for confusion rather than clearness in administration. Accordingly it would seem wiser that bacterial relations be considered in connection with keeping quality of milk and that dirt be considered as applying to such foreign matter in milk as would be ordinarily covered by that term.

It is regrettable that the conditions under which milk is produced make it inevitable that some dirt will find its way into milk. Under what would be considered exceptionally dirty conditions the total amount of dirt falling into the milk during the act of milking is rarely more than 10 mgm. per quart or 10 parts per million of the milk. Under ordinary conditions it amounts to less than one-half of this total.

The material falling into the milk is practically all dry and highly insoluble. Accordingly when later removed by mechanical means it leaves few traces of its former presence. While

this is highly desirable from the standpoint of the milk it is in a way unfortunate from the standpoint of official supervision.

The traces left by the dirt removed from the milk are the soluble portion and the bacterial life.

The soluble portion is certainly less than ten per cent of the total dirt; thus under all ordinary conditions this soluble portion would amount to considerably less than 0.5 mgm. per quart or one-half part per million of the milk. This amount is so small that thus far means for its official determination are lacking.

Much significance is usually ascribed to the bacterial life carried by the dirt falling into the milk. This impression arises largely from the distinctness with which the dirt stands out against the white background of the milk, and the consequent erroneous estimate regarding the total amount of dirt involved. Assuming that 10 mgm. of dirt fall into each quart and that each gram of this dirt carries 1,000,000,000 germs the germ content of the milk would be thereby increased by 10,000 bacteria per cubic centimeter. It should be remembered that these estimates both of the amount of dirt and of the germ content which it carries are liberal and that under average dairy conditions the germ life which milk receives along with the dirt falling into it is undoubtedly below 10,000 per cubic centimeter.

The above discussion of dirt concerns itself with the dirt falling into the milk during the act of milking because practically all of the foreign matter ordinarily designated as dirt usually enters the milk during the milking process.

Investigations have shown that practically all of the germ life introduced into the milk comes from the utensils. It is a natural supposition that this germ life upon the utensils must live upon some organic substrate and that this substrate enters the milk along with the germ life. It is undoubtedly true that occasionally dirty utensils are used in the handling of the milk. However, the effect of the use of such utensils upon the keeping qualities of the milk is so evident, and the keeping quality of the milk is so important commercially that such practices are self limiting. As a result the utensils employed in the milk industry would ordinarily on physical examination be pronounced clean, the shipping cans being perhaps the most frequent exception.

Against undesirable conditions and practices in the matter of utensils, the present available protection lies either in a determination of the germ life introduced into the milk, or in a determination of the effects of this germ life upon the keeping quality of the milk. Both of these will be discussed under the succeeding heading.

It has already been stated that while the soluble dirt in milk is slight in amount, no means for its direct measurement are available. The well known sediment test furnishes a simple means of determining the amount of insoluble dirt present in the milk, and this test can easily be made so as to give quite accurate quantitative results. As measured by this test the insoluble dirt in the milk as delivered to the consumer rarely exceeds one part per million of the milk and ordinarily is less than one-half of this amount. As delivered to the consumer, milk is ordinarily one of the cleanest foods.

An ordinance is both desirable and enforceable which requires that milk as delivered shall not contain more than some reasonable minimum of foreign matter, the exact limit being based upon a study of the local supply.

#### SWEET MILK

One of the most evident factors in the milk situation is the desire of the public for a milk supply which is not only sweet at the time of delivery, but which with ordinary care will remain sweet during at least twenty-four hours after it reaches the consumer.

This desire arises in part from the attractiveness of sweet milk as contrasted with the disagreeable flavor of milk in the early stages of souring. It also rests upon the conviction that the use of milk of poor keeping quality is intimately associated with intestinal disturbances of children.

The changes which take place in milk during the first twenty-four hours after delivery are almost exclusively the result of bacterial action. Consequently the problem of keeping quality is a problem of bacterial control.

The bacteria in milk are derived from two sources, first, direct introduction and second, growth. In the smaller cities where the milk is delivered within a few hours after being produced, the germ life in the milk as it reaches the consumer results almost exclusively from direct introduction, mainly from the milk utensils, to a far less extent from the udder flora and from added foreign matter. In the larger cities or where milk is not delivered until the lapse of twenty-four hours or more, growth may become the predominating source of bacterial life, the amount of growth being determined by the time and temperature factors.

The problem of the control of the municipal supply is complicated by the fact that both of these growth factors remain operative after the delivery of the milk and a very considerable part of the difficulty with keeping quality results from the high temperatures to which milk is exposed after it reaches the consumer. That good milk kept at blood heat will sour within twenty-four hours is a matter of common knowledge; but that keeping it at 70°F. for twenty-four hours will vastly increase its germ content, if it does not produce evident souring, is not ordinarily appreciated. Moreover few know how commonly in hot weather the family refrigerator attains a temperature of 70°F.

Much misapprehension also exists regarding the relation of pasteurization to keeping quality. The heat of pasteurization destroys not only the pathogenic germs present in the milk, but also reduces the number of the non-pathogenic germs more than 98 per cent. The number of the latter which survive is, however, roughly proportional to the number present before heating. Accordingly as a means of minimizing the action of bacteria upon milk, both before and after pasteurization, there is much to be said in favor of establishing fair standards for milk about to be pasteurized. Following the heating process the milk is exposed to various utensils including the bottles in which it is finally placed. It not infrequently happens, especially in connection with unsupervised pasteurization, that pasteurized milk has no better keeping quality than the raw milk which was originally pasteurized. In such cases its claim of better quality rests solely upon the fact that in it the probability of the presence of pathogenic germs has been removed.

The problem of simple and applicable standards of keeping quality is one regarding which the students of the question are much divided. The most commonly considered standard is that of bacterial content. Evidently the correct bacterial standard for milk about to be delivered to the consumer would be such that at the end of twenty-four hours the milk would still be in an essentially unchanged and satisfactory condition. There is as yet no agreement as to the numerical content below which such keeping quality would uniformly result though 1,000,000 per cubic centimeter has been suggested as a limiting figure.

A very real difficulty in the enforcement of bacterial plate count standards of keeping quality is the lack of laboratory provision for making such bacterial counts. However the making of these counts may be much simplified by using the "little plate" method devised by Frost (7).

In view of the simple methods now available for measuring the other elements of milk quality an equally simple method for measuring keeping quality is especially desirable.

A suggested standard of keeping quality which combines directness and simplicity consists in holding the milk in question for twenty-four hours at some determined temperature, as 60° or 65°F., before examination. Such examination may consist of smelling and tasting or it may be extended to include titration for acid and microscopic determination of the germ life.

Hastings (8) has recently revived interest in the reductase test and there is the possibility of using it in the measurement of keeping quality.

Either of these suggested forms of standard has the merit of being more simple, giving results quicker and adapting itself more readily to local conditions than any available bacterial standard which can be applied to pasteurized milk with the possible exception of the "little plate" method of Frost.

#### PRESENT MILK SITUATION

From the preceding presentation it is evident that there are now available well established methods by the use of which the desirability of a bottle of milk can be directly determined with

regard to all the elements of quality except healthfulness and this may be reasonably guaranteed by a fairly simple system of milk plant inspection.

A good municipal milk supply is positively pasteurized and protected from later contamination, carries a record of its fat content upon each bottle and is true to label, has a dirt content below some reasonable minimum and will remain sweet and in good condition for twenty-four to forty-eight hours after delivery.

Simple and reasonable as these standards may seem there are few if any municipal milk supplies which fully comply with them.

In the larger cities the requirement of positive pasteurization is generally enforced but the precautions against later contamination are in most instances only fairly well or poorly observed. In the medium sized and smaller cities even pasteurization is frequently lacking and the milk supply is correspondingly unsafe.

Not only is a record of the richness of the milk upon the bottle caps lacking in practically all cities but the composition of a very considerable fraction of the milk now retailed in these cities is not fully up to their present minimum legal limits.

The cleanliness of the milk now delivered in practically all cases is so nearly satisfactory that if attention was specifically directed to the exceptions they would undoubtedly rapidly disappear.

The keeping quality of the general milk supply is ordinarily good except for the warmer portion of the year when trouble is often experienced both with raw and with pasteurized milk. In the latter case the trouble is usually due to a failure to provide adequate protection from seeding with bacteria after pasteurization.

#### ORDER OF IMPROVEMENT

Healthfulness is at once an extremely important element of milk quality and at the same time the one which the consumer can rarely determine for himself. Accordingly a requirement that all milk shall be safe is the first object of milk control. Since tuberculosis is probably the disease most commonly spread through milk, in small communities the attempt will often be

made to safeguard the milk by insisting upon the health of the cows. In practice, this procedure will be found both expensive and difficult of satisfactory supervision and at best is only a partial solution of the problem of healthfulness. Spreading from the larger to the smaller communities there is a growing agreement that the first logical step in the protection of a milk supply is to require positive pasteurization and protection from subsequent contamination.

Reasonable standards of cleanliness are acceptable to all parties and present no particular difficulties in enforcement.

The rapidly growing interest in the question of milk standardization leads naturally to the requirement that each bottle carry a statement of its richness. When the facts are thus presented to the consumer on every package he will establish through his purchasing preference a prevailing composition of milk for each market.

In the past the sharpest conflicts of opinion have centered around standards of keeping quality. Much of the anxiety at this point is needless because the consumer quickly notes and resents any lack of this quality and the retailer appreciates that if the milk does not remain sweet his customer will be lost. The bacterial count, the time and temperature test and the reductase test may be successfully used in milk control provided they are applied with fairness and common sense.

#### CONCLUSIONS

It is the aim of this paper to point out that the milk consumer is primarily interested in the richness, safety, cleanliness and sweetness of his milk supply. Accordingly organizing the official municipal control so as to determine the food value, healthfulness, cleanliness and keeping quality will furnish the consumer with precisely the information desired regarding the milk. This information may be obtained accurately and with a minimum of expense through an inspection of milk plants coupled with the application of simple and well established tests to bottles of milk as they are delivered to the consumer.

## REFERENCES

- (1) HARDING, H. A. BREED, R. S., STOCKING, W. A., AND HASTINGS, E. G.: What is meant by "quality" in milk. Jour. Dairy Sci., i, 199-217, 1917.
- (2) PARKER, H. N.: City Milk Supply, p. 371.
- (3) HARDING, H. A.: Publicity and payment based on quality as factors in improving a city milk supply. N. Y. Agr. Exp. Station Bul. 337, 1911; also in N. Y. Agr. Exp. Station Ann. Rept., xxx, 31-66, 1912.
- (4 and 5) Definitions essentially as suggested by Dr. W. H. Price.
- (6) OVERMAN, O. R.: Food values and dairy products.
- (7) FROST, W. D.: A rapid method of counting bacteria in milk and other richly seeded materials. Jour. Amer. Med. Asso., lxvi, 889-890, 1916.
- (8) HASTINGS, E. G.: Comparative value of quantitative and qualitative bacteriological methods as applied to milk with especial consideration of the methylene blue reduction test. Jour. Dairy Sci. ii, 293-311, 1919.



# THE CAUSE AND CONTROL OF "BUTTONS" IN SWEET-ENED CONDENSED MILK

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The so-called "buttons" which are occasionally found in condensed milk are reddish-brown masses of curd, usually regular in their outline and resembling, as their name indicates, a button in general appearance. The consistency is cheesy and is sufficiently firm to allow them to be removed from the surface of the milk on which they float and to be washed free of the thick milk. They may be as small as  $\frac{1}{4}$  inch in diameter, but the typical button has a diameter of about  $\frac{1}{2}$  inch and may be as large as  $\frac{3}{4}$  inch. This description does not apply to the reddish lumps which are sometimes observed in cans sealed with solder. These are caused by drops of flux which are sometimes forced through so that a small amount of the material gets into the milk, producing a discoloration and a lump of curd. These can be usually distinguished from the real buttons by their irregular shape and softer consistency.

The general appearance of well developed buttons is shown in plate 1e. They are known to occur only in sweetened milk, and are found in both skim and whole milk, and in stored bulk goods as well as in the canned product. We have no data on the extent of the trouble or the amount of damage it causes, but it is evidently something which may occur in the product of any factory at any season of the year.

The milk itself is not seriously injured either in flavor or in food value, but the appearance of the can when opened is objectionable and would cause its rejection as spoiled by most consumers.

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**THE CAUSE OF BUTTONS**

Buttons are usually supposed to be caused by molds; in fact, it is not unusual in the industry to refer to them as "mold buttons." So far as we know, however, there is no record prior to the experiments here described of any actual proof of connection between mold growth and the buttons.

Sometimes a slight fuzzy appearance may be observed on the surface of a button, and microscopic examination not infrequently reveals mold-like hyphæ on the surface or in the interior of the button. On the other hand inoculations from the buttons to media suitable for the propagation of molds usually fails to produce any growth. This is probably due to the age of the material usually available for this purpose. The knowledge that canned milk contains buttons ordinarily comes only when the defective milk is returned by the dealer.

An outbreak of the trouble in a factory under our control gave us an opportunity to secure an abundant supply. By opening cans of various ages we were able to secure buttons in different stages of development, from simple mold colonies to typical buttons from which all evidence of the mold had disappeared. From the early stages of these buttons we obtained mold cultures which in controlled inoculation experiments produced typical buttons.

In these experiments considerable difficulty was experienced in controlling the conditions so that all of the uninoculated checks were free from mold colonies, and many of the earlier sets were invalidated for this reason. Even in milk condensed and cooled in flasks with all the usual precautions against infection many of the check cans would show mold colonies and thus render any conclusions of doubtful validity. This difficulty was finally overcome by transferring the condensed milk to small Erlenmeyer flasks which had previously been cotton-plugged and dry-sterilized. The milk in these containers was carefully heated in a water-bath to a temperature of 60°C. (140°F.) and held for thirty minutes. In this way the mold spores were destroyed without seriously affecting the physical condition of the milk. After inoculation from agar cultures the cotton plugs were replaced with sterile

rubber stoppers which were sealed in with a rubber cement used successfully in high-vacuum work. It is very essential that the supply of air be limited to that contained in the small space between the milk and the top of the container.

A typical example of an experiment of this kind is given in table 1. In this case six uninoculated flasks were held as checks and six were inoculated with a culture obtained from a typical button which had been identified by Miss Church of the Bureau

TABLE 1

*Results of examination of condensed milk in sealed flasks inoculated with Aspergillus repens*

DAYS	RESULTS
Inoculated flasks	
5	Three flasks have small mold colonies
8	Four flasks have well developed sporulating colonies
10	Fifth flask has 2 small colonies
12	Opened one flask and made agar culture
25	Colonies beginning to show reddish yellow color
31	All colonies have distinct reddish color
74	Five of six flasks have reddish colonies from which nearly all hyphae have disappeared. Characteristic buttons, but not so thick nor so sharply defined as typical buttons found in old cans
Check flasks	
74	Clean white surface. No mold colonies or buttons

of Chemistry as *Aspergillus repens*. These flasks were all held in a dark locker at room temperature.

The culture obtained on the twelfth day was found to be identical with the inoculated organisms.

From this and many other observations of a similar nature we may conclude that the button is due to the growth of a mold colony on the surface of the milk. It is apparent that the life of the mold is short and that the button is in the nature of a by-product of the growth itself. The button is made by a hardening of the casein, probable through enzym action, and continues to

develop after the mold colony has ceased to grow. We have often observed that when there is a leak in the can, even the most minute pin hole, the growth of the mold continues until the surface of the milk is covered with a felt-like growth. Under these conditions there can be no true buttons formed. In perfect cans, observations which will be reported elsewhere lead us to believe that the oxygen is almost if not quite used up in about two weeks. Since the molds are strict aerobes, growth must cease before this time. The mold hyphæ slowly disintegrate until in the old and typical button all evidence of the mold colony has disappeared.

The development from a mold colony just beginning to form spores to a button giving no indication of mold growth is shown in plate 1. The time required for the developemnt of the various stages shown in these photographs probably varies with the temperature, amount of air available, and possibly other factors. The mold colony usually appears in five to ten days. Growth probably ceases in two to three weeks on account of the exhaustion of the air. In one month the reddish-brown discoloration is quite evident and at the end of two months the button has usually assumed definite form. The disintegration of the mold hyphæ is a slow process and may not be complete before 5 or 6 months. The photographs shown in plate 1e were taken when the milk was 9 months old.

Molds of various species obtained from different sources, particularly empty condensed-milk cans, have been used in inoculation experiments, and while most of these have grown fairly well on the condensed milk and some have produced discolored spots, *Aspergillus repens* is the only one known to produce typical buttons. It is not impossible that other molds may produce buttons under favorable conditions, but the frequency with which we have isolated *Aspergillus repens* from buttons and our failure to produce buttons with others lead us to believe that this mold is the usual if not the only cause.

## THE CONTROL OF MILK

*Exclusion of contamination.* The molds and their spores are killed at a comparatively low temperature. According to Thom and Ayers<sup>2</sup> the spores of nearly all species of molds are killed by thirty minutes exposure to moist heat at 140°F., while the same exposure to 145°F. killed the spores of all but three of the large number of species tried. *Aspergillus repens* was one of these three, but the statement is made that the three molds surviving are found only occasionally in milk. They would probably be killed by the temperature of the vacuum pan, and most certainly by the forewarming. The contamination, then, comes between the pan and the can sealer.

Mold spores are very light and float in the open air even more readily than bacteria, but the moist air and clean walls and floors of the better plants are probably quite free from molds. The cans, however, are exposed to dust at various stages of their manufacture and shipment, and are almost always used without any attempt at sterilization. It might be expected that practically every can would be contaminated with button-forming molds, but the freedom of the greater part of condensed milk from buttons indicates that the can is not a serious source of contamination. Although we have examined a large number of cans, we have not isolated *Aspergillus repens* in a single instance.

The factory in which these experiments were made is so arranged that it was difficult to protect the condensed milk from mold infection. The pan was located in an open space extending from the second floor into an attic used for storage purposes. Notwithstanding these unfavorable conditions, by exercising unusual precautions we were able to protect the milk from contamination during the cooling and filling, and produce milk entirely free from buttons. It is probable that in properly constructed plants preventive measures of this kind will be sufficient to insure a high degree of freedom from buttons.

<sup>2</sup> Effect of pasteurization on mold spores. Jour. Agric. Res., vi, 153-166. 1916.

*Low temperature.* It is always possible by low-temperature storage to inhibit changes brought about by microorganisms. Many molds grow slowly at reduced temperatures even in refrigerators held near the freezing point, provided moisture and other conditions are favorable. However, the mold which we have found producing buttons grows very poorly at temperatures of 20°C. (68°F.) or lower. We have never observed buttons on milk held at 20°C. or below. It is hardly practicable under commercial conditions to store the finished product for any length of time in cold storage, and this method can not be considered as a solution of the problem.

*Exclusion of oxygen.* Buttons may be entirely and certainly prevented by taking advantage of the fact that molds grow only where a liberal supply of oxygen is available. This has been demonstrated by holding condensed milk in glass flasks sealed while under a vacuum and also in cans sealed while held in an evacuated bell jar. In this latter experiment baby-sized cans were filled with condensed skim milk containing 26 per cent milk solids from which the air had been partially removed by allowing the milk to flow slowly into a large flask held under a vacuum of about 27 inches. After filling, the milk was inoculated from agar cultures of *Aspergillus repens* and the cap soldered on, the vent being left open.

The cans were placed, one at a time, under a bell jar connected with a manometer and a vacuum pump provided with stop cocks which made it possible to maintain the vacuum at any desired point. When the vacuum had been maintained at the predetermined point for about a minute the vent was sealed by means of an electric soldering iron so arranged that it could be operated without breaking the vacuum. These cans were held at room temperature, and two cans from each lot were examined at the end of two months. The results of this examination are given in table 2.

A better idea of the general appearance of this milk is given in plate 2. The discoloration shown in the upper part of the can sealed under a 20-inch vacuum was probably caused by the flux used in soldering the cap. While this photograph shows that

the growth of mold was entirely prevented by a vacuum of 20 inches it fails to bring out the remarkable difference in color produced probably by some chemical change to which oxygen is essential. The cans sealed under atmospheric pressure had a comparatively thin layer of a dirty yellowish appearance. On the other hand the cans sealed under a vacuum of 25 and 26½ inches preserved the original appearance of the freshly condensed milk. Between these extremes were gradations in color in direct relation to the extent of the exhaustion of the air.

The perfection of a machine which would seal cans under vacuum on a commercial scale would make it possible, excluding

TABLE 2  
*The condition of condensed milk sealed under vacuum*

PRESSURE WHEN SEALED	CONDITION OF SURFACE	COLOR OF SURFACE OF MILK*
<i>inches</i>		
Atmos- pheric	Small half-developed buttons	Pale orange yellow
10	Very small buttons; one can has well developed button on margin	Warm buff
15	3 or 4 small, half-developed buttons; one has slight discoloration and undeveloped mold colony	Maize yellow
20	Small discoloration on margin of one can	Naples yellow
25	No mold or discoloration	Cream color
26½	No mold or discoloration	Cream color

\* According to Ridgeway's standard colors.

leaking cans, to entirely eliminate buttons or other defects due to the growth of molds. It is possible also that changes produced by oxidation would be materially reduced.

#### SUMMARY

"Buttons" are hard, reddish-brown lumps of curd occurring on sweetened condensed milk.

They are caused by the growth of *Aspergillus repens* and possibly other molds. The development of the mold colony is restricted by the exhaustion of the oxygen in the can and the button

itself is probably due to enzym action continued after the death of the mold.

Molds are destroyed in the process of condensing milk, and the contamination causing buttons occurs after the milk leaves the pan. Careful attention to sanitation of the plant, especially protection against dust, should be effective in excluding the greater part of the contamination.

Buttons do not develop in milk held at 20°C. (68°F.), but cold storage is probably not practicable under commercial conditions.

Molds do not grow in an atmosphere deficient in oxygen, and sealing the cans under a vacuum of 20 inches or more is an effective means of controlling buttons.



## PLATE 1

FIG. 1a. SHOWING TWO MOLD COLONIES JUST BEGINNING TO SPORULATE

FIG. 1b. THE MILK IS SLIGHTLY DISCOLORED UNDER THE MOLD COLONY

FIG. 1c. THE COLOR IS DEEPER BUT THE MOLD IS STILL VERY EVIDENT

FIG. 1d. THE DISCOLORATION IS MORE MARKED, THE BUTTON IS THICKER SO THAT IT IS SLIGHTLY ELEVATED ABOVE THE SURFACE OF THE MILK, AND THE MOLD IS DISAPPEARING

FIG. 1e. TYPICAL BUTTONS OF DIFFERENT SIZES FROM WHICH ALL SIGNS OF MOLD HAVE DISAPPEARED

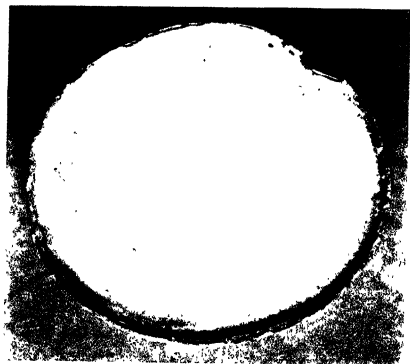


FIG. 1a

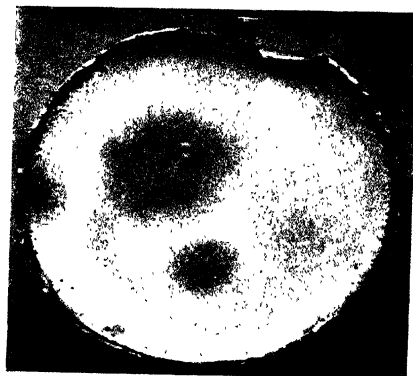


FIG. 1b

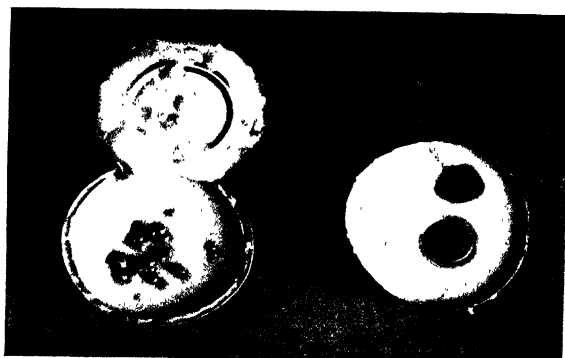


FIG. 1c



FIG. 1d

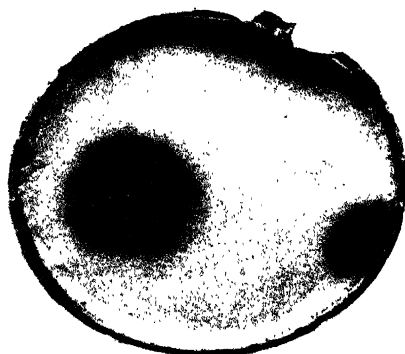
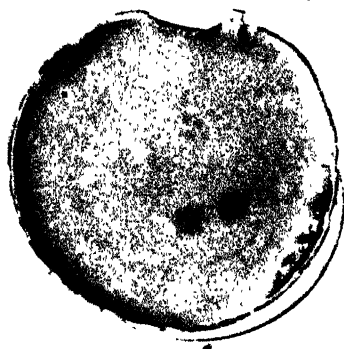


FIG. 1e

## PLATE 2

EFFECT OF AIR ON THE DEVELOPMENT OF BUTTONS AND ON THE COLOR OF THE MILK.

THE DARK SPOT ON THE CAN SEALED UNDER A 20 INCH VACUUM WAS PROBABLY CAUSED BY FLUX USED IN SEALING THE CAN.



15" Vacuum



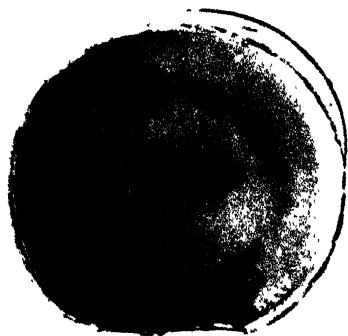
26  $\frac{1}{2}$ " Vacuum



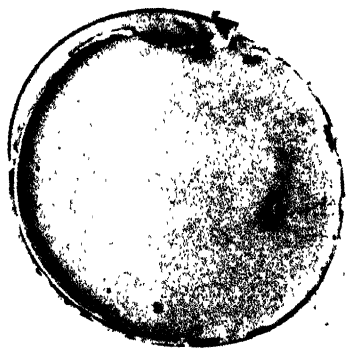
10" Vacuum



25" Vacuum



Atmospheric  
pressure



20" Vacuum

# CORRECTION FOR VOLUME OF PRECIPITATE IN THE POLARIMETRIC DETERMINATION OF LACTOSE IN MILK

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For many years the official method of the association of Official Agricultural Chemists for the polarimetric determination of lactose in milk (1), which is widely used for this purpose, has called for the dilution of the samples to 102.6 cc. for polarization in instruments equipped with the Ventzke sugar scale. 100 cc. is the volume of liquid on which polarimetric determinations are based. The additional 2.6 cc. dilution is doubtless intended as a correction to offset the volume occupied by the precipitated fat and protein in the 66 (65.96) grams of milk taken as the sample.

According to Richmond (2), the specific gravity of milk fat is 0.93 and of the milk proteins 1.346, and the corresponding specific volumes are fat 1.075 and proteins 0.743.

Leffman (3) assigns the value 1.076 and 0.8, respectively, as the specific volume of the precipitated milk fat and milk protein; and suggests their use in calculating the actual volume of the precipitated fat and proteins in correcting the polarimetric readings.

There are on file in this laboratory several hundred determinations of lactose, in securing which the volume of dilution was taken at 100 cc. and a correction applied, using Leffman's figures for the specific volume of the milk fat and milk protein and the percentage of each as actually determined in the respective samples.

Following are the formulae and conditions used in this connection:

*Readings taken on Ventske sugar scale*

Weight of milk sample taken.....	66 (65.96) grams
Dilution of precipitated sample.....	100 cubic centimeters
Length of observation tube.....	200 millimeters
Observed rotation corrected for blank reading.....	"R." of formula
Specific volume of milk fat.....	1.075
Specific volume of milk protein.....	0.8
Per cent protein.....	6.38 times per cent total nitrogen
Volume of precipitate V is 66 (1.075 times per cent fat, plus 0.8 times	
6.38 times per cent nitrogen) which is, 0.71 times per cent fat plus	
0.34 times per cent nitrogen	

$$\text{Per cent lactose} = \dots\dots\dots \frac{R}{2} \text{ times } \frac{100 - V}{100}$$

The determinations of fat were made in duplicate according to the Babcock volumetric method and the nitrogen was determined by the Gunning-Arnold modification of the Kheldahl method.

The percentage of fat and of total nitrogen and the volume occupied by the fat and protein, as calculated for 100 of our samples are shown in detail in table 1. By substituting this value in the formula it will be readily apparent that when the fat content of the milk exceeds 3.66 per cent, the volume it alone will occupy is greater than 2.6 cc., the total correction called for in the official method. In every one of the 100 samples studied at this time the combined volumes of the fat and protein exceeded the 2.6 cc. correction. The smallest discrepancy 0.6 cc. occurred with sample H 223, and the largest 5.53 cc. with sample H 18. The average discrepancy was 2.59 cc. This means, assuming the correctness of the factors used in the calculation, that an average error of 0.13 per cent would have been made if the official method had been followed in determining the lactose in these samples. The value 0.93 for the specific gravity of butter fat used in these calculations agrees very closely with the published figures of other authorities. Few figures regarding the specific gravity or specific volume of the milk proteins are available. Using Richmond's value of 0.743 as the specific volume of the milk proteins instead of 0.8 as proposed by Leffman (3) and used in computing table 1, the calculated volume of the precipitates would be reduced by amounts ranging from 0.1 to 0.2 cc.

TABLE 1

SAMPLE NUMBER	FAT	TOTAL N	VOLUME FAT	VOLUME PRO- TEIN	SUM OF VOLUME OF FAT AND PROTEIN
	<i>per cent</i>	<i>per cent</i>	<i>cc.</i>	<i>cc.</i>	
H 1	7.7	0.613	5.47	2.08	7.55
H 2	4.4	0.535	3.12	1.82	4.94
H 3	3.6	0.480	2.56	1.63	4.19
H 4	5.1	0.576	3.62	1.96	5.58
H 5	2.8	0.400	1.99	1.36	3.35
H 6	6.5	0.558	4.62	1.90	6.52
H 7	4.2	0.550	2.98	1.87	4.85
H 8	5.4	0.600	3.83	2.04	5.87
H 9	5.6	0.653	3.98	2.22	6.20
H 10	3.7	0.484	2.63	1.64	4.27
H 11	5.8	0.601	4.12	2.04	6.16
H 12	3.7	0.445	2.63	1.51	4.14
H 13	4.2	0.520	2.98	1.77	4.75
H 14	3.8	0.429	2.70	1.46	4.16
H 15	3.9	0.623	2.77	2.12	4.89
H 16	6.0	0.584	4.26	1.99	6.25
H 17	5.9	0.600	4.19	2.04	6.23
H 18	8.1	0.698	5.75	2.38	8.13
H 19	6.2	0.633	4.40	2.15	6.55
H 20	3.6	0.504	2.56	1.71	4.27
H 21	3.9	0.584	2.77	1.99	4.76
H 22	3.6	0.466	2.56	1.58	4.14
H 23	5.6	0.630	3.98	2.14	6.12
H 24	4.6	0.772	3.27	2.62	5.89
H 25	7.5	0.635	5.33	2.16	7.49
H 26	8.5	0.601	6.04	2.04	8.08
H 27	5.4	0.635	3.83	2.16	5.99
H 28	5.5	0.591	3.91	2.00	5.91
H 29	7.1	0.614	5.04	2.09	7.13
H 30	4.3	0.599	3.05	2.04	5.09
H 31	5.1	0.560	3.62	1.90	5.52
H 32	4.6	0.683	3.27	2.32	5.59
H 33	5.0	0.592	3.55	2.01	5.56
H 34	3.6	0.394	2.56	1.34	3.90
H 35	2.9	0.441	2.06	1.50	3.56
H 36	5.8	0.561	4.12	1.91	6.03
H 37	4.3	0.354	3.05	1.20	4.25
H 38	4.9	0.580	3.48	1.97	5.45
H 39	5.3	0.560	3.76	1.90	5.66
H 40	3.2	0.436	2.27	1.48	3.75
H 125	3.8	0.376	2.70	1.28	3.98
H 126	5.3	0.608	3.76	2.07	5.83
H 127	3.1	0.383	2.20	1.30	3.50

TABLE 1—Continued

SAMPLE NUMBER	FAT	TOTAL N	VOLUME FAT	VOLUME PRO- TEIN	SUM OF VOLUME OF FAT AND PROTEIN
	<i>per cent</i>	<i>per cent</i>	<i>cc.</i>	<i>cc.</i>	
H 128	3.25	0.425	2.31	1.45	3.76
H 129	5.95	0.596	4.22	2.03	6.25
H 130	3.7	0.469	2.63	1.59	4.22
H 131	3.1	0.426	2.20	1.45	3.65
H 132	5.35	0.645	3.80	2.19	5.99
H 133	5.2	0.571	3.69	1.94	5.63
H 134	3.2	0.435	2.27	1.48	3.75
H 135	6.0	0.643	4.26	2.19	6.45
H 136	5.2	0.536	3.69	1.82	5.51
H 137	3.7	0.431	2.63	1.47	4.10
H 138	3.1	0.429	2.20	1.46	3.66
H 139	3.5	0.410	2.49	1.39	3.88
H 140	5.4	0.598	3.83	2.03	5.86
H 141	5.6	0.624	3.98	2.12	6.10
H 142	5.1	0.499	3.62	1.70	5.32
H 143	5.55	0.561	3.94	1.91	5.85
H 144	3.6	0.515	2.56	1.75	4.31
H 145	3.9	0.434	2.77	1.48	4.25
H 146	3.6	0.453	2.56	1.54	4.10
H 147	5.8	0.594	4.12	2.02	6.14
H 148	5.5	0.633	3.91	2.15	6.06
H 149	5.1	0.530	3.62	1.80	5.42
H 150	4.45	0.479	3.16	1.63	4.79
H 151	7.2	0.815	5.11	2.77	7.88
H 152	3.55	0.439	2.52	1.49	4.01
H 153	5.5	0.604	3.91	2.05	5.96
H 154	5.2	0.592	3.69	2.01	5.70
H 222	3.4	0.696	2.41	2.37	4.78
H 223	2.6	0.397	1.85	1.35	3.20
H 224	3.3	0.490	2.34	1.67	4.01
H 225	3.1	0.460	2.20	1.56	3.76
H 226	3.7	0.510	2.63	1.73	4.36
H 227	3.3	0.472	2.34	1.60	3.94
H 228	3.8	0.651	2.70	2.21	4.91
H 229	5.8	0.627	4.12	2.13	6.25
H 230	3.0	0.470	2.13	1.60	3.73
H 231	5.1	0.586	3.62	1.99	5.61
H 232	5.2	0.589	3.69	2.00	5.69
H 233	3.8	0.605	2.70	2.06	4.76
H 234	3.6	0.544	2.56	1.85	4.41
H 235	3.1	0.465	2.20	1.58	3.78
H 236	4.9	0.644	3.48	2.19	5.67



TABLE 1—*Concluded*

SAMPLE NUMBER	FAT	TOTAL N	VOLUME FAT	VOLUME PRO- TEIN	SUM OF VOLUME OF FAT AND PROTEIN
	<i>per cent</i>	<i>per cent</i>	<i>cc.</i>	<i>cc.</i>	
H 237	5.2	0.681	3.69	2.32	6.01
H 238	4.6	0.531	3.27	1.81	5.08
H 239	4.6	0.566	3.27	1.92	5.19
H 240	3.3	0.463	2.34	1.57	3.91
H 241	4.4	0.615	3.12	2.09	5.21
H 242	4.9	0.616	3.48	2.09	5.57
H 243	5.0	0.609	3.55	2.07	5.62
H 244	5.7	0.466	4.05	1.58	5.63
H 245	4.7	0.544	3.34	1.85	5.19
H 246	5.4	0.562	3.83	1.91	5.74
H 247	3.0	0.497	2.13	1.69	3.82
H 248	4.8	0.649	3.41	2.21	5.62
H 249	5.2	0.620	3.69	2.11	5.80
H 250	5.8	0.771	4.12	2.62	6.74
H 251	3.2	0.485	2.27	1.65	3.92

which would increase the calculated percentage of lactose by amounts ranging from 0.005 to 0.01 per cent, and reduce the discrepancy between this method and the official method to that extent. The volume of fat and protein calculated by this formula on each of the groups of samples reported in tables 2 and 3 bear out the same conclusions, that the allowance for volume of precipitate in the official optical method for lactose is inadequate and that results obtained by its use will average about 0.13 per cent too high, the range observed being from 0.03 to 0.28 per cent. In only one small group of samples those testing less than 2.5 per cent of fat was the correction 2.6 cc. as great as the calculated requirement.

The amount of calculation involved in correcting the polarimetric readings in accordance with this formula as well as the lack of figures regarding the protein content of the milk under examination may, under certain conditions, constitute legitimate objections to its use. To meet these objections, while at the same time providing a method which will overcome the evident gross inaccuracy of the official method, the following procedure suggests itself. The writer has shown (4) that the protein con-

tent of normal milk can be estimated with a rather close degree of approximation when the fat content is known. Haecker (5) has also reported similar data. These results are summarized

TABLE 2

NUMBER OF SAMPLES IN GROUP	RANGE IN PERCENT OF FAT	AVERAGE FAT PERCENTAGE OF GROUP	AVERAGE PRO- TEIN CONTENT OF GROUP	VOLUME OF FAT AND PROTEIN IN 66 GRAMS OF MILK, COMPUTED FROM GROUP AVERAGE LEFFMAN'S FAC- TORS
15	Less than 2.5	2.19	2.61	2.43
78	Beween 2.5 and 3.0	2.76	2.80	2.91
169	Between 3.0 and 3.5	3.25	3.00	3.32
163	Between 3.5 and 4.0	3.75	3.39	3.81
97	Bewteen 4.0 and 4.5	4.25	3.52	4.22
87	Between 4.5 and 5.0	4.75	3.71	4.63
91	Between 5.0 and 5.5	5.22	3.78	5.00
52	Between 5.5 and 6.0	5.81	4.13	5.53
42	Between 6.0 and 7.0	6.47	4.46	6.10
13	Over 7.0	7.40	4.70	6.84

807 (Total samples included in study)

TABLE 3

*Percentage of fat and protein, summarized from Haecker's results*

AVERAGE FAT PERCENTAGE OF MILK	CORRESPONDING PROTEIN CONTENT	COMBINED VOLUME OF FAT AND PROTEIN
2.5	2.55	2.65
3.0	2.68	3.04
3.5	2.81	3.45
4.0	3.08	3.89
4.5	3.27	4.31
5.0	3.45	4.72
5.5	3.65	5.15
6.0	3.82	5.56
6.5	4.12	6.02
7.0	4.22	6.40

in tables 2 and 3. The volume of precipitate shown for the respective groups being used as the corrections for all samples, whose fat content placed them within the group. The correction as obtained in this way by reference to the fat content could

be applied equally well by increasing the dilution of the sample to the proper amount, as indicated for each group in column 5, table 2, or the correction could be applied as  $V$  in the formula: per cent lactose equals  $\frac{R}{2}$  times  $\frac{100 - V}{100}$ , where the samples are diluted to a uniform volume of 100 cc.

To test the accuracy of the use of these or similar figures as a basis for calculating the volume of the fat and protein in other

TABLE 4

DESCRIPTION OF GROUP, PER CENT OF FAT	VOLUME OF FAT AND PRO- TEIN COMPUTED FROM GROUP AVERAGE, TABLE 2	VOLUME OF FAT AND PROTEIN FOR 100 SAMPLES, FROM TABLE 1 CALCULATED INDIVIDUALLY. AVERAGED BY GROUPS	DISCREP- ANCY	AVERAGE DISCREP- ANCY	NUMBER SAMPLES IN GROUP
	cc.		cc.	cc.	
Less than 2.5	2.43				
Between 2.5 and 3.0	2.91	3.27	0.44	0.36	2
Between 3.0 and 3.5	3.32	3.85	1.46	0.53	15
Between 3.5 and 4.0	3.81	4.31	1.03	0.50	21
Between 4.0 and 4.5	4.22	4.84	0.99	0.62	7
Between 4.5 and 5.0	4.63	5.45	1.26	0.82	9
Between 5.0 and 5.5	5.00	5.72	1.01	0.72	21
Between 5.5 and 6.0	5.53	6.11	1.21	0.58	15
Between 6.0 and 7.0	6.10	6.44	0.44	0.34	4
Over 7.0	6.84	7.71	1.29	0.87	6
Total.....					100
Grand average discrepancy weighted for number of samples in each group.....					0.61

samples of milk, the samples studied in table 1 were grouped in accordance with their fat content. The volumes of the fat and protein as calculated for the individual samples were averaged for the respective groups. These group averages were then compared with the volume of the precipitate as calculated for the various groups of like fat percentage reported in table 2. The results of the comparison are shown in table 4.

In each group the average volume of the fat and protein is greater in case of the 100 samples reported in detail in table 1.

This group of determinations was made on individual twenty-four-hour samples from about 30 different cows at different times during the year. The cows were about equally divided between the Jersey and Holstein breeds, and were well fed and cared for at all times. The feed included pasture in season. The samples were selected from our files by groups without regard to their composition. The 807 samples, table 2, which furnish the basis of comparison for the others were obtained mostly, but not entirely, from cows of the same two breeds. A large part of the samples were twenty-four-hour composites. Another large portion were seven-day composites, while a few represented single milkings. Most of the samples were obtained from cows being used on long continued experiments, and confined exclusively to dry, and in many cases monotonous feeding but otherwise well cared for. The samples reported by Haecker were taken during the winter from well-fed cows, some of which were on high and others low-protein rations. They show a slightly smaller calculated volume of precipitate than either of the corresponding groups reported by the writer. Practically the same methods of analysis were used, but the factor used to convert Nitrogen to protein in Haecker's tables is not stated. If the commonly used factor 6.25 was employed instead of the factor 6.38 used by the writer, a part of this difference is accounted for. Whether the differences noted are due in any way to the treatment as outlined, or are only such as may be expected to occur between samples obtained from different sources and under different conditions is not apparent from the data at hand.

The average discrepancy of 0.61 cc. in the volume of the precipitates between the two methods of calculation as shown in table 4 amounts to an average error in the final lactose reading of approximately 0.03 per cent as compared with an average error of 0.13 per cent which occurs in using the official method. The maximum error of 1.46 cc. would amount to a final error of 0.07 per cent as compared with a maximum error of 5.53 cc., or 0.28 per cent in case of the official method.

## SUMMARY

It has been shown that in case of 100 representative samples studied the average volume of the fat and protein in the 66 grams of milk used as a sample was 5.19 cc. against a correction of 2.6 cc. allowed in the official method of the association of Official Agricultural Chemists. In every sample, the volume as actually calculated exceeded the correction called for in the official method by amounts ranging from 0.6 to 5.53 cc. The actual maximum, minimum and average discrepancy in the final lactose reading would have been respectively 0.28, 0.03, and 0.13 per cent. The actual computed volume of fat and protein is also shown to be greater than the correction called for by the Official method in each group of 10 groups aggregating 543 samples reported by Haecker and in each group except one of 10 groups totaling 807 samples previously reported by the writer.

A suggestion is made for the use of the fat content of the milk as a basis for determining the proper correction of volume when other data are not available. It is shown that the degree of accuracy obtainable by this simpler method is not as great as that obtainable by the use of the formula first discussed, but is much greater than that secured by the use of the official method in its present form.

## REFERENCES

- (1) Bulletin 107, revised, Bureau of Chemistry, U. S. Dept. of Agriculture.
- (2) RICHMOND: Dairy Chemistry, 1914, p. 73, Lippincott & Co., Philadelphia.
- (3) LEFFMAN, H. C.: Analysis of Milk and Milk Products, p. 38. Blackston, Philadelphia.
- (4) PERKINS: Monthly Bulletin for October, 1916, Ohio Agr. Exp. Sta.
- (5) HAECKER: Bulletin 140, Minnesota Agr. Exp. Sta.

## ORLA-JENSEN'S CLASSIFICATION OF LACTIC ACID BACTERIA

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A valuable contribution to our knowledge of lactic acid bacteria has been recently published by S. Orla-Jensen in "Mémoires de l'Académie Royale des Sciences et des Lettres de Danemark, Copenhagen" (Section des Sciences, 8me. série, t.V, no. 2). This study has covered a period of ten years duration and the results of cultural and biochemical reactions have been so correlated as to form the basis for classification.

A brief summary of our knowledge of lactic acid bacteria is necessary here in order to lead logically to the system proposed by Orla-Jensen. The term "lactic acid bacteria" has not been used by all authors in the same sense. While Kruse and other authors recognize as true lactic acid bacteria only those bacteria which are intimately concerned with the so-called normal souring of milk, others, notably Löhnis, include all those bacteria which produce lactic acid in milk and which are of consequence in the dairy. Some authors have considered as lactic acid bacteria all those bacteria which produce lactic acid, whether they are of importance in the dairy industry or not. The last view is now held to be obsolete and needs no consideration.

It is true that in the normal souring of milk there are reactions which are not entirely understood. However, it is reasonably certain that members of the coli-aërogenes group (the pseudo-lactic acid bacteria of Jensen) and *Streptococcus lacticus* are the chief and necessary agents in producing normal souring. Whether staphylococci contribute to the fermentation is not definitely settled, but if they do their influence is probably insignificant. It must be assumed then that the bacteria concerned in the normal souring of milk, namely the coli-aërogenes group and the streptococci, constitute the most important groups of lactic acid bacteria. These types have received considerable attention at the hands of investigators. A large number of

"species" was described in the early years of dairy bacteriology and the classification of Kruse was designed with a view to reducing the number of types previously recognized to a reasonable arrangement.

Recent studies of the group of lacto-bacilli have emphasized the role played by them in various processes, especially in the later stages of cheese ripening and the production of fermented milks. The acid produced by lacto-bacilli is chiefly lactic acid and since they appear to be of eminent importance in the dairy, their classification with the lactic acid bacteria is justified. The lacto-bacilli have not been studied as extensively as the first two groups mentioned. The obvious reason for this is the difficulty experienced in cultivation. Since they do not grow readily on ordinary media they have been frequently overlooked. The available studies have also led to a complicated nomenclature which awaits simplification.

The comprehensive classification of lactic acid bacteria proposed by Löhnis includes all types and calls for four main groups, namely: (1) the group represented by the coli-aërogenes bacteria, of which *Bacillus acidi lactici* (Hueppe) is the most important; (2) the streptococcus group, represented by *Streptococcus lacticus* (Kruse); (3) the staphylococci; and (4) the lacto-bacilli. Orla-Jensen has studied the last three groups and has subdivided them in his own way according to morphology, cultural properties, ability to ferment carbohydrates and in reference to the kind of acid produced.

The methods of investigation employed by Orla-Jensen include the following points: (1) morphology and cultural features; (2) sources of energy, nutritive material utilized and the manner of utilization; (3) temperature relations; and (4) agglutination and other specific properties. The results of the author's investigations are given in the following description in abbreviated form.

## MORPHOLOGICAL AND CULTURAL FEATURES

The true lactic acid bacteria or streptococci are gram-positive and when stained with fuchsin possess capsules which may attain considerable size when a tendency to slime formation is evident. Methylene blue frequently shows the presence of granules, especially in rod-shaped bacteria. The streptococci and lacto-bacilli, as a rule, grow better in stab culture than on the surface, because of their inclination to anaërobiosis. Surface cultures consist of a thin veil and the colonies are small, rarely more than one millimeter in diameter. The streptococci frequently show elongated forms, simulating bacilli.

## SOURCES OF ENERGY

As sources of carbon the following substances were used: the pentoses xylose and arabinose and the methyl pentose rhamnose; the hexoses dextrose, levulose, mannose and galactose; the disaccharids saccharose, maltose and lactose; the trisaccharids raffinose; the polysaccharids inulin, dextrin, soluble starch, glycogen and gum arabic; the alcohols glycerin, erythrit, adonit, mannit, sorbit and dulcit; the cycloparafin inosit; and the glucoside salicin. The true lactic bacteria, it was found, do not ferment gum arabic, erythrit and adonit and rarely dulcit and inosit, while the "pseudo-lactic acid bacteria" generally ferment these substances. All those lactic acid bacteria that ferment starch also ferment glycogen. Galactose is, as a rule, less fermentable than dextrose and levulose.

For the study of fermentation products four per cent. of carbohydrate was dissolved in the medium and chalk added in order to obtain a relatively large quantity of acid. Strains which produce pure dextro- or levo- or inactive lactic acid in milk produce the same modification, no matter which carbohydrate is used for the test. But the strains which produce a greater quantity of one active acid than of the other produce only the acid which is more readily formed when conditions are unfavorable. The production of an optical modification of lactic acid



is dependent upon a particular enzym secreted by the cell and not upon the stereo-chemical structure of the carbohydrate.

Casein furnishes an excellent source of nitrogen and paracasein, or the caseone formed from casein by rennet, is in many cases superior to casein. Meat extract furnishes as good a source of nitrogen as caseone provided it contains, as many commercial extracts of meat do, proteoses and peptones. Witte peptone also is a good source of nitrogen. The author thinks that the culture medium should contain a substance, peptone for example, which through its buffer action prevents injury to lactic acid bacteria from an increasing concentration of hydrogen ions. Ammonium salts and single amino-acids are entirely unsuitable as sources of nitrogen for lactic acid bacteria.

Proteins are decomposed by young cultures of lactic acid bacteria in small measure, but old cultures containing many dead and autolyzed cells decompose proteins more actively. This decomposition is therefore due to endoenzymes and is not carried beyond the stage at which aminoacids are formed. These are not split by lactic acid bacteria. The ripening of cheese is therefore a process of digestion which is supported by a mutual influence of lactic acid bacteria and rennet. This means that the acid produced by lactic acid bacteria favors rennet action, while the caseones resulting from rennet action enhance the growth of lactic acid bacteria.

Sodium chlorid has little or no effect on growth of lactic acid bacteria in quantities up to 2.5 per cent, while 5.5 per cent is harmful and 10.5 per cent prohibitive. Micrococci and sarcinae (tetracocci, Jensen), as for example those found in herring brine, withstand sodium chlorid up to 15 per cent.

#### TEMPERATURE RELATIONS

The amount of acid formed does not always coincide with the greatest proliferation. The same holds in regard to proteolysis. While, for example, *Tetracoccus liquefaciens* (Jensen), grows most rapidly at 30°C., it proteolyses most powerfully at 20°C. As a general rule the maximum and minimum temperatures are

dependent upon vitality rather than upon the composition of the medium. The optimum temperature of lactic streptococci is 30°C. or even lower, while that of pathogenic streptococci is 35° to 37°C. Most lactic streptococci are destroyed below 70°C. in fact only 0.01 per cent of cells survive 60°C. Some milk streptococci, however, survive heating to 75°C. for fifteen minutes.

#### AGGLUTINATION AND SOME SPECIFIC REACTIONS

Agglutination is not considered by the author of value in establishing a classification. The reduction of litmus is also uncertain as it is related in large measure to the rapidity of growth. The true lactic acid bacteria (streptococci) are wholly devoid of catalase and do not split hydrogen peroxid.

Variability of lactic acid bacteria is, as a rule, observed only as a result of degeneration. The author has never observed an organism to acquire the power to ferment a new carbohydrate, but has frequently noticed the loss of this power. Similarly some strains that produce both active modifications of lactic acid may under unfavorable conditions produce but one. The formation of slime in milk is highly variable. The author thinks that degeneration, such as is frequently observed in carrying cultures of starters, is due to seeding small quantity of culture. When large amounts are transferred the chance of preserving active cultures is much enhanced.

#### CLASSIFICATION

As a result of his studies Orla-Jensen recognizes the following genera: *Streptococcus*, *Betacoccus*, *Tetracoccus*, *Thermobacterium*, *Streptobacterium*, *Betabacterium*, *Microbacterium*, *Bacterium bifidum*.

#### *Genus Streptococcus*

Streptococci are spherical as a rule, but elongated before division. The daughter cell may be egg-shaped and the pointed ends may be at right angle to the axis of the chain. The cells group in

pairs or chains of varying length. In liquid media long-chained forms settle rapidly, leaving a clear fluid, while short chains remain in suspension for a long or short period, leaving the fluid turbid. Broth increases the tendency to chain formation, while in milk the diplococcus form predominates. Sometimes cell division is irregular and the streptococcus then simulates a staphylococcus. There are two main groups of streptococci, one which produces dextro-lactic acid with but a trace of by-products, while the other group forms levo-lactic acid with an appreciable quantity of byproducts. The first group comprises most milk streptococci and pathogenic forms. To the second group belongs *Streptococcus brassicae* (sourkrout) and *Streptococcus mesenteroides*. The latter type produces a considerable quantity of gas from carbohydrates. The first group is named *Streptococcus*, the second *Betacoccus*, from beta, the beet.

*Streptococcus lactis* is the chief representative of the genus streptococcus. Other names are: *Bacterium lactis* (Lister), *Streptococcus acidilactici* (Grotenfeld), *Bacterium lactic acidilactici* (Leichmann), *Bacterium Güntheri* (Lehmann and Neumann), and *Streptococcus Krusei*. No reason is given for adopting *Streptococcus lactis* instead of the more common appellation *Streptococcus lacticus*. It grows best at 30°C. and coagulates milk in twenty-four hours, forming 0.7 to 0.8 per cent lactic acid. It usually dissolves a small quantity of casein, although this faculty may be rapidly lost. It ferments maltose in preference to lactose; it ferments saccharose slightly or not at all; does not ferment dextrin and salicin. The hexoses dextrose, levulose, mannose and galactose are fermented. Alcohols are not fermented, excepting mannit which is attacked by some strains. It appears in diplococcus form or in short chains.

*Streptococcus cremoris* is the type that is used as starter for cream ripening. It frequently produces a slimy condition in milk and is identical with *Bacterium lactis longi* in Tättmjölk. and *Streptococcus hollandicus* in Lange Wei. It grows rapidly at relatively low temperature. It forms 0.7 per cent lactic acid in milk. It ferments lactose, while saccharose, maltose and dextrin are fermented exceptionally. Dextrose, levulose and man-

nose are fermented, but alcohols and pentoses are not attacked. *Streptococcus cremoris* has a more decided tendency to long chain formation than *Streptococcus lactis* and the chains are frequently encapsulated.

*Streptococcus mastitidis* is a streptococcus forming a connecting link between saprophytic and pathogenic streptococci. It grows best at 37°C. and rarely forms more than 0.5 per cent lactic acid. It is distinguished from *Streptococcus cremoris* by attacking saccharose and maltose almost as readily as lactose. It ferments dextrin and starch and forms an orange-colored deposit in starch media after ten days. Morphologically it resembles *Streptococcus cremoris* and its fermentation reactions are variable. It is therefore difficult to distinguish from *Streptococcus cremoris*, but it never produces slime in milk.

*Streptococcus thermophilus* is the commonest streptococcus in milk pasteurized at low temperature. It is not destroyed below 80°C. and grows most rapidly at 40° to 45°C. It produces more acid in milk than *Streptococcus cremoris*. It differs from *Streptococcus mastitidis* by feebly fermenting maltose and from *Streptococcus cremoris* by active fermentation of saccharose. It never attacks salicin, but ferments mannose slightly and forms long chains at 45°C.

*Streptococcus bovis* is common in cow manure. It grows best at 35°C. and not at 22°C. It grows well in milk and attacks casein, ferments starch, inulin and raffinose.

*Streptococcus inulinaceus* grows best at 30°C., does not peptonize casein and ferments inulin and raffinose. It forms short chains and has no capsule. Its other fermentation reactions are similar to those of *Streptococcus bovis*.

*Streptococcus faecium* occurs chiefly in human feces, but also in feces from other mammals. It grows slowly in milk, but rapidly in broth; it ferments arabinose and sometimes xylose. It generally ferments mannit and saccharose, but its fermenting power is quite variable and apparently approaches other types. It occurs chiefly as a diplococcus, rarely in short chains.

*Streptococcus glycerinaceus* ferments glycerin and is derived chiefly from cheese. It coagulates milk slowly, ferments man-

nit and sorbit and sometimes dulcit. It further ferments inosit, rhamnose and not infrequently xylose.

*Streptococcus liquefaciens* liquefies gelatin and digests casein. Otherwise it resembles *Streptococcus glycerinaceus*.

Eight additional strains of streptococcus were studied, but these could not be classified with the foregoing types.

### *Genus Betacoccus*

Betacocci are found on green vegetables and juicy roots. They enter the digestive tract of cattle with food and thence reach the milk. They are highly variable in their properties and the author has refrained from establishing species. They produce a slimy condition frequently occurring in syrup and are responsible for the fermentation of sourkrout. The optimum temperature is 30°C. or somewhat less. They generally produce levo-lactic acid and in some cases inactive acid. Gas, composed of CO<sub>2</sub> and H<sub>2</sub>, is produced from carbohydrates by some strains, but always in small quantity. The gas producing strains form slime in cane-sugar solutions. When isolated from vegetable matter they grow poorly in milk, but when isolated from dairy products they form considerable amounts of acid in milk and digest the casein in a measure. Betacocci ferment raffinose and sometimes dextrin and salicin. The fermentative power is very variable.

### *Genus Tetracoccus*

All carbohydrate fermenting micrococci and sarcinae are included in this genus and are distinguished from streptococcus by their luxuriant surface growth and absence of chain formation, although some strains may divide in a fashion to appear in short chains. They are distinctly aërobic, secrete a catalase and liberate oxygen from hydrogen peroxid. Some tetracocci are chromogenic and the pigment varies in color and intensity in different cultures, or subcultures from the same strain. The size of the cells is very variable and strains consisting of large cells may after subculture become much smaller. Many strains

liquefy gelatin and digest casein. Pathogenic tetracocci are killed at 65°C., while saprophytic ones may resist a temperature up to 75°C. The optimum temperature is about 30°C. Tetracocci are resistant to relatively high concentrations of sodium chlorid, sometimes as high as 15 per cent. They also resist concentrations of sugar which fact may account for their frequent presence in condensed milk. Some tetracocci form dextro-lactic acid, others produce inactive or levo-lactic acid. They are able to utilize some acids for food. The ability to ferment carbohydrates is very variable and is therefore not a reliable criterion for classification.

### *Genus Thermobacterium*

Thermobacteria are not killed below 75°C. and growth takes place at relatively high temperature. The optimum temperature is about 40°C. and the maximum lies about 50° or higher. They grow best in milk or media prepared from milk constituents. They usually form large quantities of acid in milk, but there are exceptions. The lactose fermenting thermobacteria from mash do not form acid in milk. The lactic acid formed is, as a rule, levo-lactic, rarely inactive lactic acid. Acetic and succinic acids are also formed. Some strains of thermobacteria form slime and still produce much acid. The ability to form slime sometimes is maintained through many generations. They decompose casein with formation of mono-amino acids and this quality explains their importance in cheese ripening. The lactobacillus of Emmenthaler cheese belongs to this group. Fermentation of carbohydrates is uncertain and variable. The thermobacteria are long rods, growing frequently in filaments especially in old cultures; they are inclined to anaërobiosis. Some strains show granules in stained preparations. *Bacillus bulgaricus* belongs to the group of thermobacteria. The common thermobacteria in milk are termed *Thermobacterium lactis*.

*Genus Streptobacterium*

The term *Streptobacterium* is selected because of the tendency of this group to form filaments. They are present in butter and cheese and in fermenting vegetable matter. There are many types with intermediate forms and separation into distinct species is therefore difficult. Two types are described by the author, *Streptobacterium casei* (Bacterium case  $\alpha$ ) and *Streptobacterium plantarum*.

*Streptobacterium casei* forms either pure dextro-lactic acid or in addition variable quantities of levo-lactic acid. Some strains which at first produce inactive acid later produce dextro-acid. *Streptobacterium casei* grows well in milk and forms about 1.5 per cent lactic acid. It forms casein and is therefore an active agent in cheese ripening. Some strains produce slime in milk, but readily lose this property. *Streptobacterium casei* ferments galactose and other monosaccharids. The filaments are long and often intertwined. The rods are frequently very short and then resemble streptococci. With methylene blue dark granules sometimes appear.

*Streptobacterium plantarum* produces usually inactive lactic acid, sometimes excess of dextro-acid and rarely dextro-acid exclusively. It is commonly isolated from potatoes and forms much acid in milk, but rarely attacks casein. Some strains produce slime in sugar agar, but not in milk. Fermentation of carbohydrates is variable and influenced by the source of nitrogen in the medium. Morphologically it closely resembles *Streptobacterium casei*.

*Genus Betabacterium*

*Betabacteria* are highly resistant to heat since most of them are not killed below 75°C. They usually form inactive lactic acid, but sometimes an excess of dextro-acid appears. Most strains develop gas consisting of CO<sub>2</sub> and H and when gas formation is intense succinic acid is produced as a byproduct. *Betabacteria* grow poorly in milk and usually do not attack casein. They frequently occur in cheese as they are able to

utilize lactate of calcium. They do not ferment salicin and alcohols, but ferment mannose slightly.

*Betabacterium caucassium* is one of the active bacteria in Kefir grains. Its optimum temperature is below 30°C. and it produces inactive lactic acid. The rods are of varying length and congregate in clusters.

*Betabacterium breve* ferments mannose, levulose and dextrose with difficulty. A few strains ferment saccharose and raffinose. The rods are short, have rounded ends and are separated. Short chains are occasionally met with in young cultures. Granules appear in several strains when stained with methylene blue.

*Betabacterium longum* has an optimum temperature of 45°C. It does not ferment arabinose, but some strains ferment xylose. It usually ferments saccharose and raffinose. Irregular swellings sometimes appear in stained cells, but granules are rarely seen.

### *Genus Microbacterium*

Microbacteria are small rods and generally do not curdle milk. The small amount of acid produced is dextralactic acid in all strains studied but one which produces inactive acid. Microbacteria are aërobic, split hydrogen peroxid, reduce nitrates to nitrites, and some liquefy gelatin.

*Microbacterium lacticum* is killed at 80° to 85°C., does not ferment raffinose and inulin, but frequently attacks starch. It occurs in small single rods and sometimes appears in coccus form. Stained with methylene blue it sometimes shows granules, simulating a streptococcus. *Bacillus acidophilus* of Finkelstein and Moro belongs to this group.

*Microbacterium mesentericum* is killed at 70°C. and produces but little acid. The rods are long, thin and often granulated.

*Microbacterium flavum* forms up to 1 per cent lactic acid from levulose and other hexoses. It forms a yellow pigment. The rods are plump and frequently form filaments. Stained with methylene blue granules appear.

*Bacterium bifidum* is the organism described by Tissier who found it to constitute the majority of bacteria in the feces of



breast-fed infants. Orla-Jensen could not confirm these findings, as he discovered no material difference in the fecal flora of breast-fed and bottle-fed infants. *Bacterium bifidum* is anaërobic, requires sugar for growth, produces dextro-lactic acid and large amounts of acetic acid.

The author of this very comprehensive work made a few experiments with some cultures to determine their value in practice. Cabbage was inoculated with cultures of *Betacoccus arabinosus* and *Streptobacterium plantarum* and the product was superior to the one obtained by spontaneous fermentation. The flavor of cheese was also advantageously influenced by the use of cultures. The milk used in the experiments made with cheese was first treated with hydrogen peroxid at 50°C. to destroy the majority of bacteria. By the use of cultures putrefactive bacteria are held in check and proteolytic processes were accelerated. In making Danish dairy cheese cultures of *Streptobacterium casei*, *Streptococcus lactis* and *Streptococcus cremoris* gave satisfactory results.

The publication of Orla-Jensen presents many more details than have been recorded in this abstract. The study was carefully planned and carried out with patience. The morphology of the types studied is illustrated by 280 microphotographs and several reproductions of cultures in gelatin showing the shape of the liquefied areas.

The question presents itself whether the classification proposed will fit in with general bacterial systematology. It may be questioned whether it is wise to increase the nomenclature of bacteria by the large number of names presented and whether morphology and carbohydrate fermentations are sufficiently constant to stand the test of future investigation. The production of certain chemical compounds by bacteria is apparently on a firmer basis than either morphology or carbohydrate fermentation. Certain types of lactic acid bacteria, for example, always produce dextro-lactic acid, others levo-lactic acid, and still others inactive acid. It is true that in some cases the formation of inactive lactic acid is not entirely constant as it depends on the production of equal quantities of the active acids, but in

these cases one of the two active acids is produced more readily than the other and this property remains constant. The relation of lactic acid to other acids produced is also fairly constant with the same type of microorganism and the composition of gas formed by some bacteria seems to be of a definite character.

Orla-Jensen's work undoubtedly marks progress in our knowledge of lactic acid bacteria. Recent work on this class of bacteria is confirmed and amplified. The true lactic acid bacteria are definitely recognized as streptococci and former classification, based on morphology, is shown to be incomplete. The work furthermore emphasizes the importance of correlating several tests when type determination is the object of the study. The author states that the composition of the medium is of paramount importance for the study of bacterial products and although the necessity of uniform culture media is becoming more and more apparent in bacteriologic work we are still seriously handicapped in this respect.

# THE KEEPING QUALITY OF MILK AS JUDGED BY THE COLORIMETRIC HYDROGEN ION DETERMINATION

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## INTRODUCTION

During the past few years many methods have been devised for determining the sanitary condition of milk or cream as it is delivered to the city plant or as it is delivered by the dealer to the consumer. Each of the methods devised has faults and limitations to such an extent that no one method can be said at the present time to have been generally adopted as a means of judging the sanitary quality of milk. The advantages and limitations of these methods have been dealt with in such an extensive manner in recent literature that it is not deemed necessary to include such a discussion in this paper. The fact that new methods are constantly being devised for determining the bacterial condition of dairy products is sufficient proof that the present methods are not satisfactory. The principal faults found with the methods now in use are (1) the results report the presence of inactive or dead organisms having little sanitary significance, (2) a trained technician is required to make many of the tests, (3) excessive cost per sample examined, (4) length of time required to make the test and (5) failure of the results to give a true index of the sanitary condition or keeping qualities of the sample tested.

The writers in studying the methods for determining the bacterial condition of milk were impressed with the need of a method which would meet the criticisms mentioned above. The valuable aid that such a method would be to condenseries, milk plants, creameries, cheese factories, and city, town and village health departments in controlling the sanitary condition of their milk supply makes this a problem worthy of study.

In hopes of finding a method which would be open to as few as possible of the criticisms mentioned above the writers have made a study of the change of reaction in broth inoculated with a small amount of the milk to be tested. The colorimetric hydrogen ion determination as recommended by Clark and Lubs (2) was used in making measurements.

It was hoped that in this way a factor might be obtained which would not represent the number of inactive or dead bacteria, or even the live bacteria present, but which would represent the actual ability of the bacteria and enzymes present to bring about changes in the milk which might make it unsuitable for the purpose for which intended.

In this way it was expected that account would be taken of the fact reported by Marshall and Farrand (3) that "About 57 per cent of the associate microorganisms when grown in combination with the specific lactic microorganisms accelerate their growth and action." This fact is not considered in measuring the bacteriological condition of milk by other methods.

#### METHODS

Preliminary studies were made with the indicators having a suitable range for this work. We first tried C. R. indicator, a mixture of China blue and rosolic acid, which has been recommended by Bronfenbrenner, Davis, and Morishima (1) for the preparation of milk or whey culture media. This indicator was not found adapted for our use because of its failure to adjust itself quickly to change in hydrogen ion concentration. Of the indicators studied by Clark and Lubs, Di bromo ortho cresol sulfon phthalein (Brom cresol purple) and Di bromo thymol sulfon phthalein (Brom thymol blue) were tried. Brom cresol purple having a range pH 5.2 to 6.8 was not found satisfactory because of its failure to cover the range to the neutral point. This indicator failed to give sharp color distinctions necessary for accurate measurements in this work. Brom thymol blue having a range pH 6.0 to 7.6 was found to give the most satisfactory readings and was adopted. As definite color changes with this

indicator are not present above pH 6.0 all readings falling beyond this were uniformly recorded as pH 5.8. This is the maximum reading found necessary for our purpose. Brom thymol blue was used in a 0.04 per cent aqueous solution.

Numerous culture media were tried but plain broth was found to give results (change in pH) which corresponded most closely with the actual keeping quality of the milk.

Plain bouillon cube broth was prepared and adjusted to a reaction of pH 7.0. Ten cubic centimeter portions of this were placed in large test tubes (6 by  $\frac{3}{4}$  inches) with 10 drops of the indicator, Brom thymol blue, and sterilized. A 0.1 cc. portion of the milk to be tested was obtained by adding 1 cc. of milk to 9 cc. of sterile water and adding 1 cc. of this mixture to the prepared tube of broth. The tubes were then incubated at 37°C. in a water bath or incubator. By means of the ring method we hope to do away with the use of pipettes entirely, thus greatly simplifying the technic and reducing to a minimum the chance of error.

Hourly observations were made to detect the first appearance of color change in the tubes. From the time the first change in color was noticed, hourly pH readings were made with the tubes cooled to 20°C. These readings were not continued beyond eight hours because it was found that tubes which did not show fermentation by this time represented milk of excellent quality.

#### EXPERIMENTAL

In order to determine the rate of change of the pH value of sterile milk due to the presence of lactic acid producing organisms, tubes containing 10 cc. of sterile milk were prepared and varying amounts of spontaneously soured milk placed in each. In this way samples of milk with the counts shown in table 1 were secured.

The spontaneously soured milk used had 200,000,000 acid producing bacteria per cubic centimeter when plated out on lactose agar. These inoculated milks were then treated as described under "Methods" to note rate of change in their hydro-

gen ion concentration due to the activity of the added bacteria. Samples of the milk were set aside at 21°C. and tasted at six hour intervals to determine the time required for each to become sour. It will be seen that the hydrogen ion concentration varied from pH 6.9 to more than pH 6.0 when the last reading was made. The initial readings as shown in this table vary but little from the original pH of the broth due to the small amount of milk added and to the fact that broth is a buffered solution.

The fact that the sample having pH reading of 6.5 at the end of eight hours required thirty hours to sour, while the sample having a reading of pH 6.1 only required eighteen hours would

TABLE 1

*Change in pH due to varying amounts of naturally sour milk inoculated into sterile milk*

SAMPLE NUMBER	ACID BACTERIA ADDED PER CUBIC CENTI- METER	COLORIMETRIC pH					CONDITION OF MILK
		Start	5 hours	6 hours	7 hours	8 hours	
1	0	6.9	6.9	6.8	6.8	6.8	42 hours, sweet
2	20	6.9	6.9	6.8	6.8	6.8	42 hours, sweet
3	200	6.9	6.9	6.8	6.8	6.7	42 hours, sweet
4	2,000	6.9	6.9	6.8	6.8	6.7	36 hours, sweet
5	20,000	6.9	6.8	6.8	6.7	6.5	30 hours, slightly sour
6	200,000	6.9	6.8	6.7	6.4	6.1	18 hours, slightly sour
7	2,000,000	6.9	6.6	6.4	6.0	5.8	12 hours, sour
8	18,000,000	6.9	6.2	6.0	5.8		6 hours, sour
9	46,000,000	6.9	5.8				6 hours, sour
10	66,000,000	6.9	5.8				6 hours, sour

seem to establish a rough law that any milk having a similar pH change might be expected to sour in approximately the same length of time. The first sample, from a market milk standpoint, might be considered satisfactory while the second sample because of the short time required for it to become sour would be unsatisfactory. The consumer usually feels that he has a right to expect market milk to be fit for the table at least twenty-four hours after delivery.

In table 2 the change in pH is compared with the bacterial count and the condition of the milk at the end of twenty-four hours.

It will be noticed that the eight hour pH reading compares in a rough way only with the bacterial counts, but quite accurately with the condition of the milk at the end of twenty-four

TABLE 2

*Comparison of pH change with bacteriological condition and condition of milk at twenty-four hours*

SAMPLE NUMBER	COLORIMETRIC pH					BACTERIA PER CUBIC CENTIMETER	ACID BACTERIA	PEPTONIZING BACTERIA	ALKALINE AND INERT BACTERIA	CONDITION OF MILK TWENTY-FOUR HOURS HELD AT 21°C
	Initial	5 hours	6 hours	7 hours	8 hours					
115	6.9	6.8	6.8	6.8	6.8	7,000	14.0	0.0	86.0	Sweet
120	6.9	6.8	6.8	6.8	6.8	10,000	30.0	0.0	70.0	Sweet
131	6.8	6.8	6.7	6.7	6.7	5,000	24.0	12.0	64.0	Sweet
130	6.8	6.7	6.7	6.7	6.7	6,000	41.0	33.0	26.0	Sweet
20		6.7	6.7	6.7	6.7	5,000	20.0	0.0	80.0	Sweet
21		6.8	6.7	6.7	6.7	35,000	14.0	0.0	86.0	Sweet
112	6.9	6.7	6.7	6.7	6.7	10,000	10.0	0.0	90.0	Sweet
18		6.8	6.7	6.6	6.6	50,000	20.0	14.0	66.0	Sweet
24		6.8	6.7	6.7	6.6	30,000	33.0	5.0	62.0	Sweet
11		6.8	6.6	6.7	6.6	17,000	12.0	2.0	86.0	Sweet
18		6.8	6.7	6.6	6.6	50,000	20.0	14.0	66.0	Sweet
14		6.8	6.6	6.5	6.5	100,000	30.0	0.0	70.0	Sweet
32		6.7	6.7	6.7	6.5	25,000	14.0	16.0	70.0	Sweet
19		6.8	6.6	6.6	6.5	60,000	8.0	8.0	84.0	Sweet
130	6.9	6.6	6.6	6.5	6.4	25,000	40.0	8.0	52.0	Slightly sour
23		6.7	6.6	6.5	6.4	120,000	25.0	0.0	75.0	Sweet
114	6.9	6.7	6.7	6.7	6.4	100,000	20.0	5.0	75.0	Sweet
121	6.9	6.7	6.7	6.6	6.4	130,000	12.0	75.0	13.0	Sweet
127	6.9	6.6	6.5	6.3	6.3	25,000	25.0	10.0	65.0	Sweet
128	6.9	6.6	6.6	6.3	6.2	30,000	26.0	0.0	74.0	Slightly sour
122	6.9	6.6	6.5	6.4	6.2	140,000	33.0	6.0	61.0	Slightly sour
131	6.9	6.6	6.5	6.4	6.2	300,000	65.0	1.0	34.0	Slightly sour
125	6.9	6.6	6.5	6.4	6.1	130,000	20.0	30.0	50.0	Sour
126	6.9	6.7	6.5	6.3	6.1	200,000	50.0	5.0	45.0	Sour
124	6.9	6.5	6.4	6.1	6.0	1,000,000	75.0	0.0	25.0	Curd
15		6.8	6.6	6.4	6.0	200,000	40.0	13.0	47.0	Slightly sour
17		6.6	6.6	6.5	6.0	1,200,000	7.0	8.0	85.0	Slightly sour
12		6.8	6.6	6.3	5.8	600,000	17.0	2.0	81.0	Slightly sour
22	6.8	6.6	6.6	6.4	5.8	480,000	21.0	5.0	74.0	Slightly sour

hours. As the table shows that any where from 13 to 90 per cent of the bacteria may be classified as "inert" it would seem that an accurate count of the number of bacteria present is of

little value in judging whether or not a milk is suitable for a particular purpose. A method which would actually measure the changes which the bacteria present might bring about under favorable conditions should be valuable.

It was shown in table 1 that a milk having an eight hour pH reading somewhere between pH 6.5 and pH 6.1, or more might be expected to sour in less than twenty-four hours. This is borne out in table 2 where all samples with readings of pH 6.2 or more are reported sour at the end of the twenty-four hour period.

In this table the bacterial count by the plate method is found to check in a general way with the pH readings. The first five samples of the table with an average change at the end of eight hours of only pH 0.1 have an average bacterial count of 6000 per cubic centimeter while the last five samples of the table with an average change at the end of eight hours of pH 1.1 have an average bacterial count of 690,000 per cubic centimeter.

In table 3 are tabulated the results of an examination of milk at a city milk plant. About forty samples were taken which varied but little from the thirteen tabulated. In this work the milk was tested as it arrived from the farm as number 1, 2, 3, etc. Duplicate samples of the same milk were again tested after twenty-four hours at room temperature as number 1a, 2a, 3a, etc.

The samples held at room temperature for twenty-four hours were in such condition that the readings at the end of five hours would have condemned them for market milk purposes. The condition of this milk could have been detected as early as the end of one hour if readings had been made at that time.

The results tabulated in table 4 show the change in keeping quality of milk as represented by change in pH due to holding at various temperatures. Ten samples of milk were taken as delivered to a city milk plant and each divided into four portions. Change in pH readings were tabulated after treating as follows: one portion tested at once and the other three portions held for five hours at temperatures of 1°C., 21°C., and 37°C. respectively and then tested.



The results tabulated in this table show that the samples held at 37°C. for five hours were in a condition which would make them unfit for market milk purposes. The samples held at 21°C. for five hours in every instance show a more rapid change than do the original samples. The samples held at 1°C.

TABLE 3  
*Comparison of new and old milk*

SAMPLE NUMBER	COLORIMETRIC pH					APPROXIMATE MICROSCOPIC COUNT	CONDITION OF MILK HELD AT 21°C.
	Start	5 hours	6 hours	7 hours	8 hours		
1	6.9	6.8	6.8	6.8	6.7	10,000	24 hours, sweet
1a	6.9	5.8					6 hours, sour
2	6.8	6.8	6.8	6.8	6.8	10,000	24 hours, sweet
2a	6.8	5.8					6 hours, sour
3	6.8	6.8	6.6	6.2	5.8	10,000,000	10 hours, slightly sour
3a	6.8	5.8					6 hours, sour
4	6.8	6.8	6.6	6.5	6.3	10,000,000	20 hours, sour
4a	6.8	5.8					Start, sour
5	6.8	6.8	6.6	6.4	6.0	10,000,000	10 hours, sour
5a	6.8	5.8					Start, sour
6	6.9	6.8	6.8	6.8	6.8	10,000	24 hours, sweet
6a	6.8	6.4	6.4	6.3	5.8		24 hours, sour
7	6.8	6.8	6.8	6.7	6.7	10,000	30 hours, sour
7a	6.8	6.5	6.4	5.8			6 hours, sour
8	6.8	6.8	6.9	6.8	6.7	40,000	30 hours, sour
8a	6.8	6.4	5.8				6 hours, sour
9	6.8	6.8	6.8	6.8	6.7	30,000	30 hours, sour
9a	6.8	6.2	6.0	5.8			6 hours, sour
10	6.8	6.8	6.9	6.8	6.8	12,000	30 hours, sour
10a	6.8	6.5	6.2	5.8			6 hours, sour
11	6.8	6.8	6.8	6.8	6.6	30,000	30 hours, sour
11a	6.8	5.8					6 hours, sour
12	6.8	6.8	6.8	6.8	6.8	12,000	30 hours, sour
12a	6.8	6.4	6.2	5.8			6 hours, sour
13	6.8	6.8	6.8	6.8	6.8	20,000	30 hours, sour
13a	6.8	6.3	6.0	5.8			6 hours, sour

in some instances show an improved condition over the original, others remain the same. The remainder show a poorer condition after storage at 1°C. for five hours.

This table shows the interesting fact that some samples of milk when held at the freezing point are actually improved in quality while others show a poorer quality as indicated by this

TABLE 4  
Change in pH due to holding at varying temperatures

	pH							
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours	8 hours
<i>Sample I:</i>								
As taken.....					6.8	6.8	6.5	6.4
5 hours, 1°C.....					6.8	6.8	6.5	6.1
5 hours, 21°C.....					6.7	6.5	6.2	6.1
5 hours, 37°C.....	6.8	6.7	6.7	6.6	6.1	5.8		
<i>Sample II:</i>								
As taken.....					6.6	6.5	6.2	5.8
5 hours, 1°C.....					6.8	6.8	6.4	5.8
5 hours, 21°C.....					6.6	6.4	5.8	
5 hours, 37°C.....	6.8	6.7	6.6	6.5	6.0	5.8		
<i>Sample III:</i>								
As taken.....					6.8	6.8	6.6	6.6
5 hours, 1°C.....					6.8	6.8	6.7	6.6
5 hours, 21°C.....					6.9	6.8	6.5	6.4
5 hours, 37°C.....	6.8	6.7	6.6	6.4	5.8			
<i>Sample IV:</i>								
As taken.....					6.8	6.8	6.8	6.8
5 hours, 1°C.....					6.8	6.8	6.6	5.8
5 hours, 21°C.....					6.9	6.8	6.5	6.5
5 hours, 37°C.....	6.8	6.7	6.7	6.7	6.6	6.5	5.8	
<i>Sample V:</i>								
As taken.....					6.7	6.6	6.4	5.8
5 hours, 1°C.....					6.8	6.5	5.8	
5 hours, 21°C.....					6.5	6.0	5.8	
5 hours, 37°C.....	6.8	6.6	6.4	6.2	5.8			
<i>Sample VI:</i>								
As taken.....					6.8	6.8	6.8	6.6
5 hours, 1°C.....					6.8	6.8	6.5	6.6
5 hours, 21°C.....					6.8	6.8	6.3	5.8
5 hours, 37°C.....	6.8	6.7	6.7	6.4	6.3	6.2	5.8	
<i>Sample VII:</i>								
As taken.....					6.8	6.8	6.8	6.8
5 hours, 1°C.....						6.8	6.8	6.6
5 hours, 21°C.....					6.8	6.8	6.7	6.6
5 hours, 37°C.....	6.8	6.7	6.7	6.7	6.6	6.5	6.2	5.8
<i>Sample VIII:</i>								
As taken.....					6.8	6.8	6.7	6.7
5 hours, 1°C.....					6.8	6.8	6.8	6.7
5 hours, 21°C.....					6.8	6.8	6.7	6.6
5 hours, 37°C.....	6.8	6.8	6.8	6.7	6.4	6.1	5.8	

TABLE 4—Continued

	pH							
	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours	7 hours	8 hours
<i>Sample IX:</i>								
As taken.....					6.8	6.8	6.8	6.7
5 hours, 1°C.....					6.8	6.8	6.5	6.5
5 hours, 21°C.....					6.8	6.8	6.4	5.8
5 hours, 37°C.....	6.8	6.7	6.7	6.5	6.1	5.8		
<i>Sample X:</i>								
As taken.....					6.8	6.8	6.7	6.2
5 hours, 1°C.....					6.8	6.8	6.5	6.4
5 hours, 21°C.....					6.8	6.7	6.5	5.8
5 hours, 37°C.....	6.8	6.6	6.5	6.0	5.8			

method. The probable explanation of this is that the bacteriological equilibrium is disturbed in some instances due to the effect of cold, and as a result the samples have either better or poorer keeping quality. Work is under way to determine the influencing factors involved.

These results plainly show the practical application of this test and its value to investigators.

In table 5 a provisional classification of the suitability of milk as judged by this test has been attempted. It will be seen that the milk in this table is divided into seventeen classes depending upon the activity of the bacteria present. The pH readings have been converted into per cent for practical purposes.

In testing milk from dairy farms samples in very bad condition may be picked out at the end of one hour, two hours, three hours, and on up to eight hours depending upon the condition of the milk, but the longer the tubes are allowed to incubate the more accurate are the results obtained—providing the reading of the pH does not go above 5.8.

The standard set up for milks for various purposes as shown in the table are tentative and can only be permanently placed after much experiment and observation.

Milk with a score of 60 is stated to be suitable for condensing but it is realized that milk for the several condensery products, may be suitable if having a slightly lower score or may require

a higher score. The same may be said of milk intended for market milk supply.

TABLE 5

*Provisional classification of milk as to use for which suited—dependant upon activity of bacteria and enzymes present*

READING	pH	SCORE	MILK SUITABLE FOR
<i>hours</i>			
1	5.8	20	Condemning
2	5.8	25	Condemning
3	5.8	30	Condemning
4	5.8	35	Skimming and butter-making
5	5.8	40	
6	5.8	45	
7	5.8	50	
8	5.8	55	
8	5.9	60	
8	6.0	65	Condensing
8	6.1	70	
8	6.2	75	Cheese-making
8	6.3	80	Milk Plant supply
8	6.4	85	
8	6.5	90	
8	6.6	95	
8	6.7	100	

## CONCLUSIONS

This method provides a simple and accurate means of measuring the activity of bacteria and enzymes present in the milk. The principal advantages over methods now in use are mentioned below.

The method does not measure the number of dead, inert, or living bacteria present in a sample of milk but does measure the ability of any enzymes or bacteria present to bring about changes.

The poorer samples of milk may be picked out at the end of one hour and the best samples given their proper grade at the end of eight hours.

The poorer the keeping quality of the milk, the sooner the results are obtainable.

The cost for material is one tube of broth for each sample tested.

There is no expensive equipment necessary.

A trained technician is not required. Any intelligent person may be trained to grade milk by this method in a few days time.

Over a hundred samples of milk may be easily examined each day if the comparator designed by Cooledge (4) is used.

These advantages should make this method a valuable aid to city milk plants, condenseries, ice cream factories, cheese factories, and city, town and village health departments.

By this method it is an easy matter to determine which of a dairy's patrons are delivering an unsatisfactory milk. Advice in regard to sanitation may then be given where it is needed without causing the ill feeling which results from giving advice to the patron delivering a good grade of milk.

Work which is under way will show the application of this method to pasteurized milk and to ice cream.

#### REFERENCES

- (1) BRONFENBRENNER, J., DAVIS, C. R., MORISHIMA, K. 1919 Jour. Med. Res., January, p. 345.
- (2) CLARK, WM. M., AND LUBS, H. A. 1917 Jour. Bacteriol., ii, Nos. 1, 2, and 3.
- (3) MARSHALL, C. E., AND FARRAND, BELL 1908 Special Bulletin No. 42, Michigan Agricultural College.
- (4) COOLEIDGE, L. H. 1920 Jour. Indus. and Eng. Chem.

## BOOK REVIEW

*Milk.* PAUL G. HEINEMANN. Philadelphia, Pa., W. B. Saunders Company.

Students of the milk problem are under obligation to Dr. Heinemann for having condensed a large amount of usable and important information into a volume of less than 700 pages. "Comprehensive" is the best descriptive term to apply to this new book dealing with a much discussed and investigated subject. Because of the range of topics covered it is sure to attract a wide circle of readers.

Users of the book gain from the fact that its author has had opportunity to observe the reaction of students to the material presented. As a result he has been discriminating and careful in the selection and presentation of material. The subject matter of the book, like the lectures on which it is based, covers the physical and chemical properties of milk, the biology of milk including bacteriology, and methods of producing and distributing milk.

The selection of titles for the bibliographies given at the end of each chapter shows the author to be familiar with the important developments in each of the fields discussed. The bibliographies are not complete, but are sufficiently so to open up the fields under consideration. A careful reading of those portions of the book with which the reviewer himself is most familiar failed to reveal any of those slips in statement of fact which are so hard to avoid in a book of this type.—R. S. BREED.



# ORGANIZATION OF INVESTIGATION IN AGRICULTURE<sup>1</sup>

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Washington, D. C.*

The organization of investigation with a view to a larger measure of coöperation among institutions and workers, and greater attempt toward coördination, is at present perhaps the livest subject connected with scientific research. Hardly an address is delivered which does not touch upon this subject, and very many in the past year have dealt primarily with it. The idea is not new, as the work of this Association testifies, but it has been given new force and far broader scope in the past few years.

This association is well-nigh a pioneer in that field, and the systematic study it has devoted to the details and means of accomplishing the ends sought has made it one of the going agencies in this line. There is something stimulating in knowing that the idea the Association has stood for is gaining ground, and we may feel a new conviction that in this effort we are on the right track and in the line of progress. It may be interesting to reflect briefly on the recent growth of efforts in this field.

The war gave a great impetus to coöperative effort in all directions, including agriculture. It developed the National Research Council, which is founded in the idea of coöperative and coördinated effort. It gave so many illustrations of usefulness that the Council has been made permanent, and is now organized on a generous plan which embraces a division for the biological sciences and agriculture. The prime purpose of the Council is to afford opportunity for coöperation and to assist in bringing it about, without itself having part in the investigations. It aims to serve as a stimulating agency, rather than

<sup>1</sup> Read at the Atlanta meeting of the Association of Southern Agricultural Workers, February 26, 1920.



to build up within itself means for carrying on research or for subsidizing it. It does not purpose to centralize the administration of research but to popularize it, to direct attention to the need for it, and to democratize efforts at organization for specific lines of inquiry. As you have seen, the Council has recently received from the Carnegie Corporation a gift of \$5,000,000, one million of which is to be used for erecting a building in Washington as a permanent headquarters of the Council and the home of the National Academy of Sciences.

The divisions of the Council embrace many committees. One of these on nutrition is to consider problems in the field of animal as well as human nutrition, and another relating to fertilizers and soils has recently undertaken to catalogue, with the aid of the Office of Experiment Stations, all the projects bearing on this field which are now under way at the experiment stations, in the Department of Agriculture, and at other institutions in this country. Such a classified list of live undertakings may serve as a basis for coöperative or coördinated attack, and it may later furnish the means for a thorough digestion of the investigation in particular lines, in order to determine what may be accepted as established and what next steps are indicated. The work of the National Research Council bears, therefore, on that of this Association, and it seems not improbable that to an extent the two may join hands.

The Association of Land-Grant Colleges has, as you know, taken a definite stand in favor of coöperation and proposed the formation of a joint committee with the Department of Agriculture, to be known as an agricultural research council. As yet but little progress has been made in consummating the idea, but the step is a most interesting one as evidencing the present attitude of experiment station workers. Various other organizations, such as the botanists, pathologists, agronomists and the chemists, are committed to the idea of encouraging organized research.

That coöperation in research of various types and grades is feasible and practical there is increasing evidence, drawn from experience in lines where it has been going on. Dr. Hale, of

the National Research Council, has called attention to a number of notable examples in astronomy, physics, engineering, geology, and chemistry. In the study of sediments and sedimentary deposits, for example, the geologist must have the help of the chemist; and, as Dr. Hale points out, "it is easy to see how an investigator choosing to deal with some aspect of this large, general problem would be assisted by information regarding related work planned or in progress, and how readily as a member of the group he could render his own researches more widely useful and significant." In connection with one of the undertakings referred to, Dr. Hale mentions that certain specifications were formulated but those who took part were not bound by any rigid rules. On the contrary, as he says, "they were encouraged to make every possible innovation in the manner of attack in order that obscure sources of error might be discovered and the highest possible accuracy in the final results obtained. The outcome demonstrates most conclusively that organized effort and freedom of initiative are by no means incompatible."

The organization of investigation logically begins with the individual station. To a considerable extent the present policy and program are largely an accumulation of the past. Hence changes may be brought about only slowly. The war led to a review of station projects and the stressing of certain ones of special importance. In a number of cases this has had a permanent effect and resulted in a revision of the entire station program. I have been much impressed with the systematic attempts at several stations to develop a program of work which would be not only more live but more definitely adapted to the special problems and needs of their localities. For example, one station appointed a committee on projects to review the whole list, ascertain the status of each project, the time it had been going, the progress it was making, its prospects and importance, and the work necessary for its completion. This committee under the supervision of the director made a catalog showing for each project the publications issued upon it, its relation to other work of the station, its adequacy, and the future plans of the leader. The latter appeared before the committee and discussed his

projects, explained their importance, and in the end recommended what ones should be continued and what should be brought to a close as soon as feasible.

The advantage of such a review must be apparent. Each project stood on its own merits. Some which had been drifting but had been using up money were detected, and their future decided. In this way a conservative and safe means was provided of sifting out the less active or important as soon as practicable, and a basis furnished for a live, active project list. You will recognize that it furnished the best possible means for establishing coöperation and coördination within and without the station.

At another station a research committee was appointed to revise the project list in much the manner referred to above, but in addition to construct a program of station work designed to better cover matters of prime importance and less dependent on chance or special preference. To this end the heads of departments, extension specialists, and county agents of the state will canvass the needs for investigation, and the suggestions presented will be weighed and incorporated in an adequate constructive program of investigation. Other stations have taken similar steps, but there are indications that the practice might well be more general.

Most stations have too many projects. They are to some extent accumulations, and represent a desire of individual workers to have a considerable list of undertakings. To this extent they embody a false idea, an ambition not in accord with the present views of investigation. It is desirable to encourage narrowing the scope in many cases, and organizing the work so that it will be more definitely centered on specific questions of limited range. The very fact that stations have forty, fifty, and even a hundred projects shows how widely their efforts are being scattered, how far they fail of concentration.

How many projects did Hellriegel have when he set about settling once for all the long contested question of whether legumes take nitrogen from the air? The famous Rothamsted station has always limited its efforts quite definitely. It has

concentrated on special questions, and has broadened its field of investigation almost entirely as a result of questions which the progress of these studies developed. Hilgard concentrated the work of his station on soils and their utilization, expanding his program only gradually as new funds came. One of the chief reasons why Hopkins met with such success in his studies was that, although they covered a quite wide range, they were definitely and consciously centered in his purpose to develop principles of soil fertility and permanent agriculture.

The specialist with only two or three projects may accomplish more for his own reputation and for the permanent benefit of practical agriculture than one who is attempting to carry a dozen or twenty, as some unfortunately are. It is an aid to the man with a long list of projects to have this list reviewed by others in a sympathetic but critical manner, and account of stock taken. It helps him to discard or terminate those he may have been doubtful about, and to direct his efforts along more constructive channels.

Such a scrutiny provides not only against a scattering of effort but against superficial, intermittent work. It may even affect the composition of the staff itself. Station staffs have been made up in the past largely on the basis of departments instead of problems. The reason for this is clear and need not be elaborated; stations have often had to make the best of the material at hand. But with a larger number of persons now assigned primarily to research, the special needs of the station and the lines it plans to study may well figure in the search for a worker and the assignment of duties. In other words, men may be sought to do definite things.

Too often a botanist or a chemist or an animal husbandman has been brought into the staff because there was a vacancy, and then asked to outline some projects, and perhaps told that as he was to be paid partly from the Adams fund he must get one or two lines that would fit that fund. More rarely perhaps is he advised of the station's program and invited to take hold of some phases of problems included in this program, to occupy at least a part of his time. How different is the case with an

industrial concern which opens research laboratories! The latter has certain problems to be worked out and is on the lookout for others in its field which are likely to prove profitable.

Such a selection of men to carry forward predetermined lines is entirely feasible in the case of station forces. When the director of the Maine Station went in search of a biologist he had a very definite purpose in view, and although the man he selected had never worked in that particular line and was not familiar with poultry raising, he was thoroughly trained, and he adapted himself to the situation, developing a department of research of national reputation. There are, of course, many similar illustrations which go to reinforce the feasibility of the station itself making and shaping the general plan of operations, and giving it direction, instead of leaving it to the various departments working independently, or making it contingent on the individual ability of the teaching staff.

It is becoming clearer with the development of station work that in large measure it should be organized around problems. In this the relations of the various departments need to be considered. Broad questions will often involve coöperation, or a division of the inquiry between departments. This may mean either a close working together, or an approach from different standpoints, or the taking up of separate but essential phases of the question. It may even involve one department working for another or under its direction for the time being.

In the drift toward specialization, scientific men have more and more segregated themselves into groups each of which confines itself to the study of a special and often narrow field. Specialization represents a great advance. It recognizes the deeper insight, the necessity of intensive study, and a differentiation of field and of skill. But specialization is opposed to generalization and may unfit men for it.

While specialization has served greatly to advance scientific knowledge, there is a danger in its isolation of retarding the solution of complex problems like those in agriculture. These problems have often been worked upon from the standpoint of the individual specialist, without particular reference to what

investigators in another branch are doing. From the standpoint of the individual a special phase and not the broad problem may become the unit. It does not necessarily require a specialist to see a problem, and he may not see it in its entirety. The analysis of a question is an important step toward its study, and such analysis often needs the combined insight of specialists in different fields. Hence the advantage of organization of research around problems in such a way as to unite this viewpoint and means of attack.

Some men are more resourceful in planning and conducting investigation than others. There are some who are natural leaders, and others who do their best work in association. It is the business of the director to determine this and to use his force and facilities to the best possible advantage. It is one of his functions to study the workers and their work, to determine whether the latter is progressing as it should, to ascertain its needs if there are weak points, and to provide help from another department where it is required. He should form a judgment of the members of his staff, and until he has that there is little warrant for authorizing large undertakings. We ought to avoid experimenting with men as far as possible.

Economy of the supply of workers, and especially those of outstanding ability in research, suggests utilization of their talents to the utmost. Research is not alone for the few if proper guidance can be supplied. Leadership is of great importance to make most highly effective the work of the rank and file. The history of science shows to how large an extent discoveries and important deductions have rested upon long series of accurate observations requiring care and patience, but not necessarily great genius. "The method of science is not a mysterious gift of genius but a practical tool in the discovery of facts and their application to the problems of everyday life." Much credit, therefore, belongs to the patient workers whose efforts help to make discovery possible provided their work is so done that it can be knit together.

A man's inherent right to work independently depends first on his ability and his particular problems, and second on the

requirements of the station program. Such right may never mean freedom from supervision or direction. It should not be forgotten that the workers in an experiment station are members of an organization, bound together by a common interest and purpose, and subject in the final analysis to the general plan of a public service institution. This does not imply any narrow view, the sacrificing of ambition, or the subordination of individuality, but it implies loyalty to a cause and to an organization. It means, what has long been clear and freely admitted, that many of the intricate problems in agriculture are larger than any individual, and that their solution as rapidly and completely as is humanly possible is something the public has a right to expect from these institutions. It is natural therefore that stations should frequently find it desirable to combine their labors and their forces. The fact that the director and his staff constitute the experiment station, and that they themselves in very large measure determine its working program, makes it a singularly democratic institution in which loss of individuality need rarely be feared. Merit will tend to attain its proper level.

Dean Thatcher, of Minnesota, has recently made a strong appeal for the adoption of "such a real spirit of coöperation as will bring to the solution of our problems the combined results of training and experience of all the workers who can contribute anything to either the immediate progress of an investigation or its final practical application." To develop such a spirit, he describes the conference groups of scientific workers which have been established at the Minnesota Station. The purpose is to provide an opportunity for frequent discussion and friendly criticism of the methods and results of research in progress, and to insure that when any new project is being considered all phases or scientific aspects of the problem may be given due consideration and properly provided for. The result has been a very pronounced growth of the general spirit of coöperation at that station. The plan is worthy of wider introduction.

A natural effect of thoroughly organizing the work may ultimately be felt in the type of problems attacked. There will, of course, be many projects which aim at the settlement of some

single fact or local question, but more and more the type of problems to be studied will be such as relate to broad fundamental questions of permanent character and wide application, bearing ultimately on the formulation of good agricultural policy and practice. These from their nature will inevitably call for relating the work of different departments, and suggest coöperation both in attack and in interpretation. General principles, broad underlying facts, and the understanding of their limitations and controlling factors, have a far more enduring value than results which pertain to minor questions or deal primarily with local aspects or conditions.

When the work of a station has been well organized, when each house has been set in order, the way is more clear for arranging for effective coöperation or coördination between stations. This should not be on too extensive a scale, especially at the outset, and should be as free as possible from complications and cumbersome machinery. A small number of undertakings are more likely to succeed than if the attempt is made to bring a large part of the work into coöperation. A few things well and satisfactorily done are more important than many less effective ventures. It is by success in coöperative effort that the plan will win friends and conviction, and will grow by its own force if the means are provided.

For most of the experiment stations the organization of their work with reference to what others are doing is no longer a matter of preference alone, but in a greater measure is impressed upon them by present conditions. Insufficient funds make such action necessary if the stations are to cover the field and render the service expected of them. Individual workers and separate stations have their quite distinct limitations, and their efforts may be materially supplemented and strengthened by those of other institutions. It should be realized that joint effort is a means of making the work of each station and each individual worker more effective.

The American stations comprise a system having a common purpose, as well as local responsibilities, and confronted with many problems common to regions extending far beyond state



boundaries. The stations do not exist merely to themselves or for their states. They have a unity of purpose, and a range of interest which is not confined to the local aspects of problems. They are interdependent. They can economize their time and funds, and make their efforts more productive of sound conclusions by so relating their work as to cover certain problems completely, make the attack more concentrated, and the results more readily comparable or intelligently harmonized.

Coöperation may lead to a more intensive study of the nature of the problem—what it really involves, what features or branches of science are included in its manifestations, and hence the means of approaching it, instead of viewing it from a one-sided, individualistic standpoint, or in the practical form in which it comes up to the station. One great need is a more careful definition of problems. In agriculture they are unusually complex, and the factors they embrace are often quite obscure. We are apt to see these problems in their composite character, as involved, practical questions, rather than in their fundamental aspects. Attempts to solve them in such form really aim at providing empirical rules for farming and usually lead to results which are themselves largely empirical.

Coöperation and correlation may result in minimizing duplication. Repetition is all right if warranted, but we all know there has been a vast amount of going over similar ground in a similar way without adding anything new that is material or contributing to the final solution. There is, of course, little reason for duplication which is not constructive and fails to take account of what has gone before.

Then again the lack of coöperation and better understanding has had an unmistakable effect on the public. Fragmentary, inconclusive and discordant results have led to criticism and lack of confidence. There is still need to inspire the confidence of the public, to create the feeling of dependence on the teachings of experimentation, and above all to avoid confusing the farmer and his teachers.

If the extension workers are to bank on experiment station results and conclusions, the latter must be well fortified and

beyond the controversial stage when they are given out for popular consumption; we should agree fairly well among ourselves, or understand why we do not agree without reservations. I think the station bulletin is an improper place for criticism and controversy. These are appropriate in their place, but their proper place is the scientific journals and the meetings of specialists. In the station bulletin they are likely to be misinterpreted and to weaken the position of investigators.

One essential to joint effort is the provision of conference of those involved or disposed to join hands. Nothing can take the place of this in organizing work, and in analyzing and determining what should be covered in the solution of a problem. Coöperation must be a democratic effort. A centralized, made-to-order plan, to be followed rigidly by those who participate, starts off wrong. Coöperation should begin with the outlining of the problem and the making of the plan. It is in this that the different views and breadth of knowledge of facts and conditions are of great advantage. Out of the exchange of ideas and the discussion of the real nature of the question at issue there should come more carefully digested and effective plans for attack.

Finally the plan should preferably be as simple and elastic as the subject will permit, leaving as much latitude as possible to individual initiative, preference, and ingenuity. The whole effort should be informal, but at the same time it should not be without coherence and follow-up, or it will disintegrate into unrelated, independent effort.

The most important thing at this time, therefore, is to recognize the advantage and develop the spirit of coöperation. The next is to provide the opportunity and means for it. There needs to be a wider knowledge of what is being done and where, such as you have attempted to provide, and there should be free intercourse among those engaged in common undertakings. I think it is becoming apparent that we have less and less to fear from unworthy competition as a result of exchange of ideas, and more and more to gain from combined and coördinated effort.

# MILK PRICES AND COST OF MILK PRODUCTION

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## INTRODUCTION

The present study pertains to the seasonal cost of producing milk for direct consumption. Numerous organizations in various parts of the United States have made from time to time many studies of the cost of milk production, but in most instances these data involve what is known as the year cost of milk production. Little or no attention has been given to the seasonal variations in the cost of producing this important commodity.

The question of seasonal variations in the cost of fat production was discussed briefly in 1914 before the American Farm Management Association and later the proceedings were published by the association.<sup>1</sup> The present study presents quantitative results which set forth in rather precise form the large variation in the cost of producing milk for urban consumption in the different months of the year.

## SOURCE OF DATA

The Department of Dairy Husbandry has been carrying on detailed cost-accounting investigations pertaining to various problems of dairy farm administration since 1912. The data presented in this bulletin involve eighteen of the thirty-six farms included in the study of the year cost of milk production published as Bulletin 216 of this station.<sup>2</sup>

<sup>1</sup> Proceedings of 5th Annual Meeting of American Farm Management Association.

<sup>2</sup> Pearson, F. A. The Cost of Milk Production Computed on Year Basis. 1919, p. 24.

## COW COST OF MILK PRODUCTION BY MONTHS

The cow cost of milk production involves only the maintenance of the bulls and the milking stock; the cost of rearing the young stock is not included. The milking herds are maintained by purchasing cows or by replacing depleted stock with heifers raised in the herd.

The data presented in table 1 involve 407 cows and 19 bulls, the cows producing 2,806,277 pounds of milk at a net cost of \$48,479.67, or \$1.73 per hundredweight. It will be noted that the net winter cost of producing this milk was \$31,669.94, or \$1.98 per hundredweight, and the summer cost, \$16,809.73, or \$1.40 per hundredweight. The monthly cost varied from \$1.15 per hundredweight in June to \$2.08 in December and February.

## VARIATION IN FEED EXPENSE

For the herds under consideration the feed expense per hundredweight of milk produced, varied from 40.1 cents in June to \$1.41, or over three times as much, in December, with an average of \$1.05 for the year. For the winter months the feed expense aggregated \$21,307.29, or \$1.33 per hundredweight of milk, and for the summer months, \$8,159.56, or \$0.68 per hundredweight of milk.

## VARIATION IN LABOR EXPENSE

The expense of man labor per hundredweight of milk produced varied from 27.9 cents in June to 41.0 cents in December, with an average of 36.3 cents for the year. During the winter six months an average of 11.7 hours per day of man labor was spent on each herd, while in the summer months an average of only 7.9 hours per day was used.

While the variations exhibited in the labor expense are not so great as those shown in feed expense, they are still great enough to contribute materially to the large variation in the total cost of production.



	SUMMER MONTHS					
	May	June	July	August	September	October
Milk production in pounds .....	284,867	239,145	190,701	149,785	140,108	198,942
Items of expense:						
Feed other than pasture .....	\$1,379 37	\$638 17	\$758.21	\$880.60	1,053 82	\$1,633 43
Pasture .....	285 07	331 05	297 91	290 98	328 62	292 33
Man labor.....	826.43	666 36	654.41	606 01	590 92	719 07
Live-stock depreciation ..	601 23	504 73	402.49	316 13	295 71	419 88
Use of buildings ..	205 02	172 12	137 25	107 80	100 84	143 18
Horse labor.....	204 33	161 46	156 35	150 20	146.42	178 24
Interest on stock .....	197 68	197 68	197 68	197 68	197 68	197 68
Miscellaneous ..	176 65	148 29	118 25	92.88	86 88	123 36
Use of equipment.....	35 61	29 90	23 84	18.73	17.52	24 87
Total.....	\$3,911 39	\$2,839 76	\$2,746 39	\$2,661 01	\$2,818 41	\$3,732 04
Returns not milk						
Manure.....	\$189 93	\$33 26	\$41.39	\$45 82	\$66 51	\$193.63
Calves.....	139 55	48 54	103 14	133 48	424 71	479 31
Total.....	\$329 48	\$81 80	\$144 53	179 30	491.22	672 94
Net cost .....	\$3,581 91	\$2,757 96	\$2,601 86	\$2,481.71	\$2,327 19	\$3,059.10
Net cost per hundredweight .....	1 26	1 15	1 36	1 66	1 66	1 54

## QUANTITIES OF FEED AND LABOR PER HUNDREDWEIGHT OF MILK

By an inspection of table 2 it will be noted that with the cow as the unit of computation, the value of the man labor, grain, hay, dry forage, and succulent feeds represents 77.8 per cent of

TABLE 2

*Cow cost of milk production: Amounts of man labor and feed other than pasture required to produce 100 pounds of milk, and the percentage which the value of these forms of the net cost*

	YEAR	WINTER MONTHS						
		January	February	March	April	November	December	Winter, six months
Man labor (hours) . . . . .	2 29	2 39	2 43	2 39	2 26	2 42	2 56	2 41
Feed								
Grain (pounds) . . . . .	32 4	41 5	51 6	48 3	39 8	40 0	41 7	43 9
Hay (pounds) . . . . .	36 4	58 0	52 5	47.1	43 2	60 1	59 1	53 0
Other forage (pounds) . . . . .	25 9	49 1	41 1	35 6	24 6	55 9	49 4	42 1
Silage (pounds) . . . . .	154 0	204 2	183 4	154 7	162 8	183.8	214 6	183 2
Percentage that feed and labor form of net cost .	77 8	88.1	87 0	84 2	83.1	88.0	87 1	86 3
		SUMMER MONTHS						
		May	June	July	August	September	October	Summer, six months
Man labor (hours) . . . . .		1 85	1.76	2 18	2 58	2 69	2 28	2 15
Feed								
Grain (pounds) . . . . .		20 6	13 7	13 4	12 9	15 5	26 7	17 6
Hay (pounds) . . . . .		14.9	1 7	8 6	13 8	20.0	29 9	14 2
Other forage (pounds) . . . . .		0 78	0 29	0 37	0.48	8 7	17 8	4 3
Silage . . . . .		79 3	49 9	91.4	192.5	227.4	130 5	115 0
Percentage that feed and labor form of net cost . . . . .		61.6	47.3	54 3	59 9	70.7	76.9	61 9

the net yearly cost of producing the milk. The percentage which feed and labor constituted varied from 47.3 in June to 88.1 in January.

The amount of labor per 100 pounds of milk varied from 1.76 hours in June to 2.69 hours in September, averaging 2.29

hours for the year. The grain fed per 100 pounds of milk varied from 13.7 pounds in June to 51.6 pounds in February, averaging 32.4 pounds for the year.

The amounts of feed and hours of labor, and the percentage which the total value of these forms of the net cost, being determined, it is relatively simple to determine the approximate cost of milk production for any given period. The costs being expressed in commodities may be converted into terms of value by applying farm prices to these units.

Assuming the prices are as follows the June cost would be:

Grain . . . . .	13 70 pounds at \$55 per ton =	\$0 377
Silage.....	49 90 pounds at 6 per ton =	150
Hay . . . . .	1 70 pounds at 10 per ton =	009
Other roughage.....	29 pounds at 6 per ton =	001
Man labor . . . . .	1 76 hours at 25 cents per hour =	440
Value of feed and labor . . . . .		\$0.977
Proportion that feed and labor form of total net cost...	47 3 per cent	
Net cow cost of milk per hundredweight . . . . .		\$2 07

Figured in a similar manner, the December cost would be \$3.30 per hundredweight of milk; the summer cost, \$2.34; the winter cost, \$3.19; and the year cost, \$2.81.

#### SIGNIFICANCE OF SEASONAL VARIATIONS IN FEED AND LABOR EXPENSE

In the past due significance has not been attached to the rôle that pasture plays in dairy farming. The most popular method of dispensing with the pasture question has been to contrast the total amount of food secured from an acre of pasture with the amount secured from an acre of corn silage or alfalfa. However, in spite of much advice to the contrary, the dairy farmers have continued to make general use of pasture since the cheapest food for the summer months is obtained in this way and the farmer is enabled to reduce the amount of labor spent on the herd. As the months favorable to pasturing stock approximately coincide with the crop season, this reduction in the amount of labor spent on the herd enables the farmer to spend more labor on the crops.



On the eighteen farms under discussion, the man labor devoted to the production of milk during the summer months totalled 27,632.5 hours, an average of 152 hours per day. In the winter months it amounted to 43,533.0 hours, or 238 hours per day. The difference per day per farm between summer and winter was 4.8 hours.

The expense for feed in the summer months is much less than in the winter months. In this study, in the month of June, when pastures are best, the feed expense was considerably less than one-third the average for the winter months. This was due to the fact that much more grain was fed per hundred-weight of milk in the winter months than in the summer months.

Pasture is a crop which is in most cases planted, grown, and harvested without much assistance from the farmer. Dairy farmers pasture-feed in the summer, rather than barn-feed, because they usually have in permanent blue grass some land which cannot be tilled with profit. Pasture reduces the amount of labor spent on the herd and permits more time to be spent with the crops during a period when the returns secured from labor spent on crops are relatively large compared with returns from the herd. If the farmers did not pasture their stock in the summer and yet continued in the dairy business, barn-feeding during the summer months, more buildings would have to be maintained to house the additional feed necessary, or fewer cows would have to be kept. Farmers without pasture who embark in the dairy business and sell to the ordinary markets have one of two alternatives: either they must barn-feed in the summer, or they must turn good crop land into pasture. The first alternative, in the absence of a large amount of unpaid labor, is very unprofitable. The latter, although unprofitable, is probably less so than the former.

#### SEASONAL VARIATION IN COST OF PRODUCTION AND THE PRICE OF MILK

The price of a commodity must in the long run cover the cost of production or production will be diminished. Milk is no exception to this rule. Furthermore, the price of a commodity

like milk for urban consumption, which is both bulky and perishable, must fluctuate approximately with the cost of production or production will be concentrated in the more profitable seasons.

The accompanying figure presents graphically the monthly percentage variation in the price paid for milk at the Chicago market during the ten years 1907 to 1916<sup>3</sup> and in the cost of production with the cow as the unit.

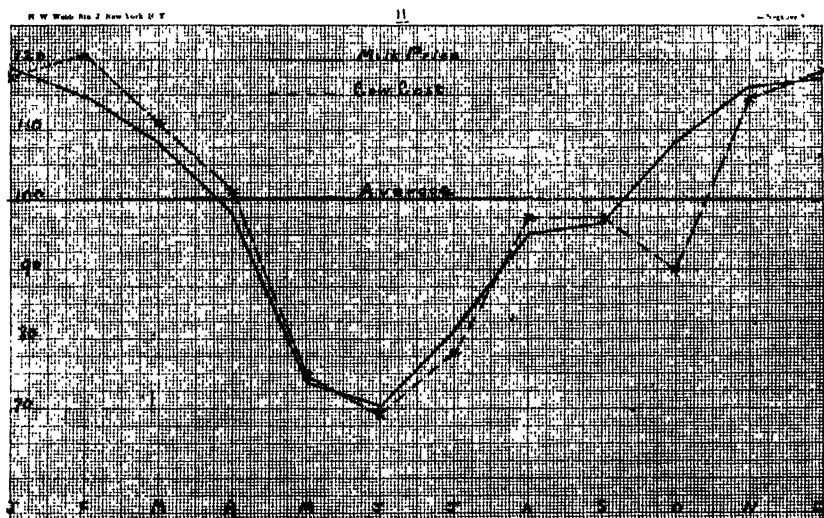


FIG. 1

These curves indicate that in general the monthly variation in the price of milk has followed more or less closely the monthly variation in the cost of production. The price of milk tends to vary somewhat less than either the herd cost or the cow cost of production, not rising so high in the winter nor falling so low in the summer. The monthly cow cost per hundredweight of milk varies from 122 per cent in February and December to 67 per cent in June. The price of milk varies from 120.3 per cent in December to 70.6 per cent in June.

<sup>3</sup> For tabular presentation, see page 356 of Bulletin 216.

In the past many persons have recommended a flat price for milk throughout the year. This recommendation has usually been made in the absence of due appreciation of the wide variation in the monthly cost of production. A flat yearly price for milk is out of the question, as production would tend to concentrate in the summer months of low cost. This would result in a supply in the winter season that would not be sufficient to meet the demand.

Other persons, admitting that a flat yearly price is not practical have suggested that prices should be on the basis of two six-month periods, with the price for the winter months higher than that for the summer months. This scheme has many advantages over the plan of a flat yearly price, but it would still be impossible to set a price that would encourage a fairly constant volume of milk throughout the period, because the fluctuation in the cost of production is so great from month to month. For instance, April is a month during which a large volume of milk is produced at a cost considerably greater than that for the summer months, but yet somewhat lower than that for the winter months. April being between the two seasons of high and low prices, is the month during which, even under the present scheme of monthly adjustment, the farmer producing winter milk probably suffers most. Under a six-month scheme the price of April milk would have to be either as low as the summer price or else as high as the winter price. The former would be a handicap to the producer and the latter a burden to the buyer.

Again, it would not be satisfactory to sell milk in June, when there is usually a surplus, at the same price as in August and September, when there is usually a scarcity, although these months occur in what are known as the summer months. Similar discrepancies could be pointed out for the winter months.

Owing to the great variation in the monthly cost of milk production, a flat price for any extended period would probably shift production to the more profitable months. Since milk production is so sensitive to changes in prices, the milk producer, the milk distributor, and the milk consumer are best protected

through a fluctuating price, which insures, so far as it now seems possible, a fairly constant supply of milk. If the price of milk fluctuates approximately with the cost of production, the distributor's supply is automatically regulated, the milk producer's market is protected, and the consumer is assured of a normal supply of milk throughout the year.

### CONCLUSIONS

1. The amount of man labor involved in the production of milk is considerably less in the summer months than in the winter months. This is true whether based upon the total amount of labor used on the herd or whether based upon the amount involved in the production of 100 pounds of milk. Proper significance of this reduction in labor is appreciated only when attention is drawn to the fact that these savings in labor occur during the pasture season, which coincides with the crop season, when the maximum labor is needed in the field.

2. Aside from man labor, feed, and horse labor, the expenses of producing milk are more or less constant throughout the year. When all expenses are included, the net cost of producing 100 pounds of milk in June is about 60 per cent of the year cost, and in December about 120 per cent.

3. With a fluctuating seasonal cost, it is to be expected that farmers will tend to concentrate production in the more profitable months. As the urban trade demands a constant supply of milk throughout the year, the price of milk must fluctuate approximately with the cost of production in order to prevent an extreme shortage at one time and a large surplus at another. In other words, a properly adjusted fluctuating price for milk throughout the year protects the farmer's market and the distributor's and consumer's supply.

# A MODIFICATION OF THE HAECKER AND SAVAGE FEEDING STANDARDS FOR DAIRY CATTLE

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The feeding standards have now come to be recognized as valuable adjuncts to the teaching of the principles of feeding and as criteria by which rational feeding methods can be outlined. However, consideration must be given to the fact that no feeding standard can ever be looked on as determining definitely the amounts of nutrients that must be supplied to certain animals, for the simple reason that the individuality of an animal is a very variable factor and at the same time is the chief one in determining the feed requirements of the animal. In addition there are certain factors in nutrition, such as the variations in the quality of the proteins; the occurrence and distribution of the "vitamines," and the requirements of the animals for ash, that are not considered in feeding standards. In spite of these drawbacks the feeding standards are of great value and have come to stay. The present problem is to render the standards not only more nearly accurate but also more easily applicable.

All of the standards in use can be considered as modifications of the Wolff-Lehmann standard, those of Haecker (3) and Savage (5) being stated in terms of digestible nutrients while the standards of Armsby (1) and Eckles (2) are given in terms of digestible true protein and net energy. No effort will be made to discuss the accuracy or relative merits of the standards but a suggested modification of those based on digestible nutrients will be given.

The Haecker standard (3) is given in terms of digestible crude protein, digestible carbohydrate and digestible fat and the use of three units of measurement obviously renders it somewhat cumbersome. The Savage standard (5) modifies the Haecker standard somewhat and is stated in terms of digestible crude protein and total digestible nutrients, this latter term being the

sum of the digestible crude protein, the digestible carbohydrate, and  $2\frac{1}{4}$  times the digestible fat. There is a distinct disadvantage in using the two terms, digestible crude protein, and total digestible nutrients.

When a ration is calculated according to this standard it is easy to see where the protein in the trial ration meets the requirements of the standard but it is difficult to determine when the carbohydrate equivalent portion of the ration meets the requirements. For example, if the protein requirements should not be met with the trial ration and the total digestible nutrients should appear to be correct it is evident that the carbohydrate equivalent portion of the ration is incorrect and a mental calculation must be made to determine the necessary amount of carbohydrate equivalent that must be added or deducted in order to bring the ration into line with the requirements of the standard.

A still further modification has been made by Morrison (4) by simply using the Haecker and Savage protein requirements as minima and maxima respectively and giving the requirements for total digestible nutrients as the average of the requirements of the two standards. In the practical use of the standard the range in protein requirements is not used as a general rule, the common practice being to use the average of the two for protein requirements.

Consequently, the table presented here has been calculated so that the protein requirements given are the averages of those called for by the Haecker and Savage standards and the carbohydrate equivalent requirements of the two standards have also been calculated and averaged. In this way the requirements of an animal can be stated in two simple terms—digestible crude protein and digestible carbohydrate equivalent—which are independent of each other.

The requirements for the production of one pound of milk, ranging from 2.5 per cent to 7.0 per cent in butterfat content are given. The maintenance requirements for use with this table are 0.0700 pound of digestible crude protein and 0.7925 pound of digestible carbohydrate equivalent per 100 pounds live weight.

TABLE 1

*Digestible nutrients required for the production of 1 pound of milk*

FAT IN MILK	DIGESTIBLE NUTRIENTS	
	Crude protein	Carbohydrate equivalent
<i>per cent</i>	<i>pounds</i>	<i>pounds</i>
2.5	0.0459	0.1999
2.6	0.0466	0.2045
2.7	0.0473	0.2091
2.8	0.0480	0.2139
2.9	0.0487	0.2198
3.0	0.0494	0.2246
3.1	0.0501	0.2295
3.2	0.0508	0.2344
3.3	0.0515	0.2402
3.4	0.0522	0.2453
3.5	0.0529	0.2511
3.6	0.0536	0.2560
3.7	0.0543	0.2619
3.8	0.0550	0.2667
3.9	0.0557	0.2715
4.0	0.0564	0.2774
4.1	0.0571	0.2823
4.2	0.0578	0.2871
4.3	0.0585	0.2919
4.4	0.0592	0.2968
4.5	0.0600	0.3016
4.6	0.0607	0.3061
4.7	0.0614	0.3100
4.8	0.0621	0.3146
4.9	0.0628	0.3183
5.0	0.0635	0.3230
5.1	0.0642	0.3278
5.2	0.0649	0.3313
5.3	0.0656	0.3362
5.4	0.0663	0.3397
5.5	0.0670	0.3445
5.6	0.0677	0.3481
5.7	0.0684	0.3529
5.8	0.0691	0.3575
5.9	0.0698	0.3614
6.0	0.0705	0.3659
6.1	0.0712	0.3697
6.2	0.0719	0.3745
6.3	0.0726	0.3781
6.4	0.0733	0.3840
6.5	0.0741	0.3875
6.6	0.0748	0.3913
6.7	0.0755	0.3948
6.8	0.0762	0.3996
6.9	0.0769	0.4043
7.0	0.0776	0.4070

## REFERENCES

- (1) ARMSBY, H. P. 1917 The nutrition of farm animals.
- (2) ECKLES, C. H. 1913 Nutrients required for milk production. Res. Bul. Mo. Agr. Expt. Sta. 7.
- (3) HAECKER, T. L. 1914 Feeding dairy cows. Bul. Minn. Agr. Expt. Sta. 130.
- (4) MORRISON, F. B. 1915 The modified Wolff-Lehmann standards. Proc. Am. Soc. Animal Prod., p. 64.
- (5) SAVAGE, E. S. 1912 A study of feeding standards for milk production. Bul. Cornell, N. Y. Agr. Expt. Sta. 323.



# FISHY FLAVOR IN BUTTER<sup>1</sup>

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## INTRODUCTION

The improvement of the quality of butter has been the aim of investigators and manufacturers of dairy products for many years. Abnormal flavors and odors in milk, cream, butter and cheese have caused the dairyman endless trouble and annoyance. To preserve the normal flavor in butter and cheese, the efforts of the dairyman have been directed almost entirely in the direction of controlling the methods of manufacture of these products, while little attention has been given to chemical and bacteriological changes produced by the various methods used. The effect of the various methods of handling and treating milk and cream have had little interpretation as to the chemical changes produced in these products or the influence of bacterial growth. It is noted that chemical changes are produced in cream handled in certain ways, and the chemical changes in the pabulum of the bacteria must have its influence on the growth of the various types of organisms retained in the resulting butter. Butter handled one way seldom if ever goes "fishy," while to diverge from this certain method of handling the cream before churning would endanger the quality of the resulting product. The presence of a chemical substance, having a flavor and odor of decomposed fish, must necessarily be produced in the butter by synthesis or decomposition of some of the components of the cream. As the decomposition of one of the constituents of the butter would theoretically give a substance having a fishy flavor and odor, it may be concluded in the light of recent investigation, that this substance—tri-methylamine—is the cause of fishy flavor in butter. What is the rôle played by bacteria in bringing about this change is the subject of our investigation.

<sup>1</sup> This work was done in the Department of Dairy Industry, N. Y., State College of Agriculture, under the direction of Prof. W. A. Stocking.

## REVIEW OF LITERATURE

In 1900, fishy flavor and odor were found in milk by Harding, Rogers and Smith (1) at the New York Agricultural Experiment Station at Geneva. In this case the milk from a single member of a herd of cows was found to have a strong fishy odor and taste. However, the reason for the milk from this particular cow being "fishy" was not ascertained. An organism suspected of causing the abnormal flavor was isolated and various experiments performed to determine if the suspected organism would reproduce fishy flavor. No positive results were obtained.

O'Callaghan (2) (1901) declared that *Oidium lactis* was the cause of fishy flavored butter. Even unsalted butter was reputed to have developed this disagreeable flavor. His work was repeated by Rogers and also by Reakes, Cuddie and Reid (3) (1912). They all agreed that *Oidium lactis* was not the cause of fishy flavor. The latter authors conclude that:

The development of fishy flavor in butter arises as a result of a chemical change including a splitting up of some of the constituents into compounds possessing this peculiar character and taste, the factors responsible for such change being apparently of a degree of high acidity of the cream and overworking.

Harrison (4), however, agrees that fishy flavor in butter is due to the presence and growth of undesirable bacteria in cream.

Rogers (5) (1914) gave out the information that fishy flavor is usually preceded by an oily or a metallic flavor. He found that fishy flavor is not prevented by low temperatures, but only retarded by them. Also that salts of iron and copper accelerate the production of fishy odor and taste. He never found fishy flavor in unsalted butter.

In the opinion of the writer, fishy flavor is caused by the slow, spontaneous chemical change to which acid is essential and which is favored by the presence of small amounts of oxygen. Fishy flavor may be prevented with certainty by making butter from pasteurized sweet cream. Butter made from pasteurized sweet cream with starter, but without ripening, seldom if ever becomes fishy.

Hunziker (6) (1915) found that high pasteurizing temperatures, such as 185°F. and higher, may cause a very poor quality of butter. The butter seemed to have a disagreeable oily flavor suggesting also that of decomposed fish. This occurred more particularly in summer when the cows are in pasture and the butter fat contains more than the average amount of olein.

The action of high acid and high temperature on the olein, Hunziker thinks, is the cause of fishy flavor in this instance.

Hammer (7) (1917) isolated an organism from a can of evaporated milk that had a strong fishy odor and flavor. He inoculated both sterile and pasteurized milk and cream with this organism and fishy flavor developed in a few hours. In inoculated milk there was also produced coagulation and digestion. He was, however, unable to produce fishy flavor in butter either by inoculating the butter directly or by adding the culture to the cream before churning.

In some lots of butter, salt was used, while other lots were unsalted. The counts made showed that the number of bacteria per gram decreased throughout the holding period with butter made from sour cream and with salted butter made from sweet cream, while with unsalted butter made from sweet cream there was an increase followed by a decrease.

The organism was called *Bact. ichthyosmius*.

In repeating Hammer's work, butter was made from cream which had been inoculated with a culture of *Bact. ichthyosmius*, and in some cases fishy flavor developed in the butter after a few months in storage. The cause of the fishy flavor is not credited entirely to the *Bact. ichthyosmius* as the treatment of the cream before churning seems to have brought about chemical changes in the product which evidently were responsible for the development of the undesirable odor and taste in the butter.

#### PLAN OF THE EXPERIMENT

Cream containing 30 per cent butter fat was used to make the butter for these experiments, and the six portions taken treated as follows:

*Portion D.* About 750 cc. of sweet cream was pasteurized at 145°F. for thirty minutes, and then cooled to 48°F. The cream was then inoculated with 1 cc. of an aqueous solution containing a culture of *Bact. ichthyosmius* which had been grown on an agar slant. This organism was isolated from a group of ten, and was the only one to produce fishy odor in sterilized milk. The butter was made in the usual manner, one half of the portion was salted, while the other half was not. Both samples were stored in a refrigerator at 15°F. Another sample of cream was treated in the same manner as the above sample except that it was not inoculated. This latter was a check sample for D.

*Portion E.* This sample of sweet raw cream was inoculated like the preceding sample, incubated at 98°F. for three hours; cooled to 60°F. and then churned. From this point E and all of the following samples were treated the same as in sample D.

*Portion K.* The sweet cream was inoculated; incubated at 98°F. for three hours, the acidity neutralized to phenolphthalein with sodium hydroxide, and churned at 60°F.

*Portion N.* The cream here was inoculated, incubated, and neutralized as in portion K. After neutralizing, one ounce of liquid starter was added, and the mixture allowed to stand at room temperature for three hours before churning.

*Portion R.* This portion was inoculated; one ounce of starter added, and the cream allowed to stand at room temperature for three hours to ripen. At the end of three hours it was neutralized and then churned.

*Portion X.* This last sample was inoculated, incubated for three hours at 98°F.; cooled to 60°F., starter added; and churned after three hours.

Each portion of cream then was represented by an unsalted sample of butter as well as a salted sample inoculated with *Bact. ichthyosmius*, and a check sample which was not inoculated, but was salted. These were all stored at 15°F. Every two weeks, during a period of eight months, a bacteria count was made on both the inoculated samples from each portion. The colonies were grown on lactose agar. The counts noted in the portions represent the number of bacteria per gram of butter. All three samples from each portion were scored frequently for quality (flavor).

*General summary of the work of scoring.* No check (uninoculated) sample was scored "fishy." No unsalted, inoculated, sample was scored "fishy." Salted sample from portion D (inoculated), made from fresh cream which was pasteurized and then churned at once, was not scored "fishy." The bacteria decreased rapidly both in the salted and in the unsalted sample. Samples from portion E were not scored "fishy." The bacteria count on the salted sample was 120,000,000 at the time it was churned, and at the end of six months the count was 460,000. The unsalted sample had a count of only a few hundred at the end of four months. The only difference between this portion and the preceding was in the neutralization of the sample after incubation. The salted sample of portion N was accidentally destroyed at the beginning of the fourth month in storage. However, the author scored the sample "fishy" a short time before the accident. Portion R after four months in storage had a strong fishy odor. At the end of seven months the odor had disappeared somewhat. The salted sample from portion X was scored "fishy" by one of the judges, "tallowy" by another, while the two remaining judges scored it "metallic." The number of bacteria per gram of butter in this sample was several million even after it had been in storage for several months.

DATES OF PLATING	D.		D. S.	
	Bacteria per gram	Scored 10/28	Bacteria per gram	Scored 10/28
3/19	23,400,000		62,000	
4/2	18,250,000		5,000	
4/16	11,500,000		2,150	
4/30	7,500,000		2,000	
5/14	4,050,000	1. 0	1,900	1. 0
5/29	3,000,000	2. 0	700	2. 0
6/11	600,000	3. 0	500	3. 0
6/25	505,000	4. 0	200	4. 0
7/9	435,000		000	
7/24	405,000			
8/7	285,000			
8/21	45,000			

D. = Made from sweet cream, pasteurized, and churned at once.

D. S. = Same as D. except salted.

Acidity of both, 0.135 per cent.

DATES OF PLATING	N		N. S.	
	Bacteria per gram	Scored 10/28	Bacteria per gram	Scored
3/19	1,232,000,000		16,600,000	
4/2	42,000,000		8,950,000	
4/16	25,000,000		6,650,000	
4/30	20,000,000		6,400,000	
5/14	20,050,000		5,450,000	
5/29	18,800,000	1. 0	4,600,000	
6/11	18,600,000	2. 0	3,750,000	
6/25	5,800,000	3. 0	Sample accidentally destroyed	
7/9	2,850,000	4. 0		
7/24	1,780,000			
8/7	205,000			
8/21	70,000			

N. = Sweet cream, pasteurized, inoculated at room temperature, neutralized, and then ripened with B.l.a.

N. S. = Same as N. except salted.

Acidity of samples, 0.32 per cent.

DATES OF PLATING	R.		R. S.	
	Bacteria per gram	Scored 10/28	Bacteria per gram	Scored 10/28
3/19	1,600,000,000		10,400,000	
4/2	8,750,000		10,100,000	
4/16	7,800,000		8,300,000	
4/30	Plate badly contaminated with fleshy colonies after third count was made on this sample		6,700,000	
5/14			6,350,000	
5/29			4,000,000	
6/11			1,350,000	
6/25		1. 0	595,000	1. Fishy
7/9		2. 0	670,000*	2. Oily
7/24		3. 0	740,000*	3. Fishy
8/7		4. 0	455,000*	4. Fishy
8/21			370,000	
9/4			350,500	
9/26			320,000	
10/28			215,000	

\* Fishy odor on plates at time of counting.

R. = Sweet cream, pasteurized, inoculated, incubated at room temperature, neutralized, ripened with B.l.a. and again neutralized.

R. S. = Same as R. only salted.

Acidity of both samples at time of neutralization was 0.36 per cent.

Sample R. is noticed here only to compare its score with that of R. S.

DATES OF PLATING	X.		X. S.	
	Bacteria per gram	Scored 10/28	Bacteria per gram	Scored 10/28
3/19	900,000,000		32,700,000	
4/2	46,050,000		30,250,000	
4/16	31,800,000		28,000,000	
4/30	2,500,000		17,400,000	
5/14	2,160,000	1. 0	14,150,000	1. Metallic
5/29	1,950,000	2. 0	11,500,000	2. Metallic
6/11	1,810,000	3. 0	11,200,000	3. Tallowy
6/25	165,000	4. 0	6,400,000	4. Fishy
7/9	124,500		5,400,000	
7/24	56,000		4,300,000*	
8/7			4,000,000*	
8/21			4,500,000	
9/4			4,600,000	
9/26			3,200,000	
10/28			4,200,000	

\* Fishy odor on plates at time of counting.

X. = Sweet cream, pasteurized, inoculated, incubated at room temperature, and then ripened with B.l a.

X. S. = Same as X. only salted.

Acidity of samples at time of churning was 0.297 per cent.

DATES OF PLATING	K.		K. S.	
	Bacteria per gram	Scored 10/28	Bacteria per gram	Scored 10/28
3/19	52,900,000		23,400,000	
4/2	46,000,000		14,400,000	
4/16	40,500,000		10,450,000	
4/30	34,000,000		8,500,000	
5/14	27,400,000	1. 0	6,300,000	1. Metallic
5/29	26,150,000	2. 0	1,800,000	2. 0
6/11	27,100,000	3. 0	1,800,000	3. Fishy
6/25	27,900,000	4. 0	1,150,000	4. Fishy
7/9	18,600,000		890,000	
7/24	14,650,000		750,000	
8/7	7,500,000		Plates became too clouded for further study	
8/21	2,200,000			
9/4	515,000			
9/26	460,000			
10/28	Cloudy plates			

No fishy odor was detected from any of these plates.

K. = Sweet cream, pasteurized, inoculated, incubated at room temperature, and then neutralized before churning.

K. S. = Same as K. only salted.

Acidity of samples at time of neutralization was 0.45 per cent.

DATES OF PLATING	E.		E. S.	
	Bacteria per gram	Scored 10/28	Bacteria per gram	Scored 10/28
3/19	27,200,000		120,500,000	
4/2	11,450,000		43,250,000	
4/16	6,100,000		22,950,000	
4/30	2,800,000	1. 0	17,000,000	1. 0
5/14	2,330,000	2. 0	5,050,000	2. 0
5/29	850,000	3. 0	2,950,000	3. 0
6/11	785,000	4. 0	2,910,000	4. 0
6/25	170,000		1,990,000	
7/9	50,000		1,225,000	
7/24	19,000		1,205,000	
8/7	000		990,000	
8/21			1,030,000	
9/4			1,005,000	
9/26			460,000	
10/28			241,000	

E. = Sweet cream, pasteurized, inoculated, incubated at room temperature before churning.

E. S. = Same as E. only salted.

Acidity of both samples before churning, 0.45 per cent.

In some of the samples of the experiment, fishy flavor was produced by inoculating the cream with *Bact. ichthyosmius*. The handling of the cream before churning affected the growth of the bacteria differently in every portion. To show how the samples of butter are affected chemically by the different methods of handling the cream, tables 1, 2, and 3 are given here showing a series of butter made from cream which was handled in various ways and which are comparable to the ways in which the cream portions of the inoculation experiments were treated. For instance, portion D compares very favorably with portion 6 of table 1 as to the way it was treated before churning. Portion 6 retained a large amount of its total phosphorus, and the soluble organic phosphorus suffered little, if any, decomposition. Portion E may be compared with portion 2 of table 1. The total phosphorus in 2 is very high, and the soluble organic phosphorus is large in amount. The portions which were scored "fishy" K. X. and R. underwent treatment similar to portion four (4) in table 1. This portion four (4) showed a very small amount



of organic phosphorus in the organic form, and it may be inferred that some of its organic phosphorus (soluble) had been decomposed. By comparison, all three samples (K. X. and R.) prove that where there is a loss of soluble organic phosphorus, fishy flavor develops in the butter. The only possible organic phos-

TABLE 1  
*Salted samples of butter made 1/11/18, analyzed 1/28/18\**

BUTTER FROM CREAM PORTION NUMBER	PER CENT OF $P_2O_5$				
	Soluble inor- ganic	Total soluble	Soluble or- ganic	Protein resi- due	Total
1	0.0129	0.0156	0.0027	0.0123	0.0338
2	0.0138	0.0168	0.0030	0.0108	0.0397
3	0.0100	0.0124	0.0024	0.0079	0.0249
4	0.0127	0.0132	0.0005	0.0131	0.0351
5	0.0170	0.0202	0.0032	0.0103	0.0414
6	0.0155	0.0180	0.0025	0.0095	0.0344
7	0.0108	0.0124	0.0016	0.0089	0.0300

\*From "Phosphorus in Butter" by this author.

TABLE 2  
*Analyses of above samples April, 1919*

BUTTER FROM CREAM PORTION NUMBER	PER CENT OF $P_2O_5$				
	Soluble inor- ganic	Total soluble	Soluble or- ganic	Protein resi- due	Total
1	0.0158	0.0204	0.0046	0.0120	Totals checked
2	0.0232	0.0236	0.0004	0.0163	
3	0.0191	0.0193	0.0002	0.0086	
4	0.0157	0.0168	0.0011	0.0163	
5	0.0227	0.0242	0.0015	0.0166	
6	0.0201	0.0211	0.0010	0.0115	
7	0.0148	0.0207	0.0059	0.0091	

TABLE 3  
*Unsalted sample portion 2 of above butter*

	PER CENT OF $P_2O_5$				
	Soluble in- organic	Total soluble	Soluble or- ganic	Protein residue	Total
2. Analyzed 1/28/18.....	0.0158	0.0199	0.0041	0.0139	0.0468
2. Analyzed 4/4/19.....	0.0229	0.0250	0.0021	0.0147	

phorus which is readily soluble in salt solution is lecithin. . Butter contains lecithin, and one of the decomposition products of lecithin is choline. This choline is decomposed giving trimethylamine which has the odor and flavor of decomposed fish. Undoubtedly in the treatment of cream in certain ways, the lecithin is broken down to a form which is used as pabulum specifically by the Bact. ichthyosmius.

At the end of the period of storage the organism was isolated from a sample of butter which had been scored "fishy," and its identity determined.

### *Morphology*

Form: rod shaped.

Size:  $1\mu$  to  $2\mu$  long, and from  $0.6\mu$  to  $0.8\mu$  wide.

Arrangement: isolated.

Motility: actively motile.

Staining reaction: Gram negative.

Spore formation: none found.

### *Cultural characteristics*

Agar streak: dirty-white growth, non-viscid.

Agar stab: dirty white growth on surface and throughout the entire length of the stab.

Agar plate colonies: ellipsoidal colonies, white by reflected light and brown by transmitted light.

Milk: peptonized.

Litmus milk: peptonization and production of acid.

### *Bio-chemical features*

Gas production: gas in bouillon containing sucrose, but not with lactose.

Indol: positive tests in bouillon and in milk.

All the above characteristics are in perfect agreement with those found by Hammer for Bact. ichthyosmius.

As may be seen from the counts on the different samples of butter, the number of bacteria decreased rapidly, in most cases,

in the unsalted samples and in the salted samples which did not go "fishy," while in the salted samples which did develop a fishy flavor the decrease in the numbers of bacteria was gradual. No unsalted samples were scored "fishy," nor has fishy flavor been found in unsalted samples of butter in any of our work. The check samples which were not inoculated with *Bact. ichthyosmuis* but which were treated in every way as those described in the data given above, were kept in every instance, and in no case was fishy flavor observed in any of them. Only three of the samples were scored "fishy," and the methods of handling the cream before churning were similar in all three cases. The limiting factor in the production of fishy flavor in butter seems to be in the apparent decomposition of one or more of the constituents of butter during the preparation of the cream before churning and this decomposition product is used as pabulum by bacteria. Lecithin, a constituent of butter, from its chemical constitution can be broken down to a compound which has the odor and flavor of decomposed fish. *Bact. ichthyosmuis* may specifically utilize the decomposition products of lecithin and bring about the formation of tri-methylamine, the compound which tastes and smells like decomposed fish.

#### CONCLUSIONS

1. Fishy flavor was produced in butter by inoculating the cream with *Bact. ichthyosmuis*.
2. The age of the cream, the acid content of the cream, and the period of incubation before churning seem to influence the production of fishy flavor in butter.
3. Decomposition products of lecithin apparently are used as pabulum by *Bact. ichthyosmuis* and the organism forms therefrom tri-methylamine giving fishy flavor to the butter.

The author wishes to express his thanks publicly to the Dairy Department for furnishing material for this problem and to Prof. W. A. Stocking for suggestions and aid in carrying out the work. Also to Professor E. S. Guthrie, Dr. G. C. Supplee, H. C. Jackson and W. E. Ayres for scoring the butter used in this problem.

## REFERENCES

- (1) HARDING, H. A., ROGERS, L. A., AND SMITH, G. A. 1900 N. Y. Agr. Experi. Sta., Geneva, Bul. 183, pp. 179-181.
- (2) O'CALLAGHAN, M. A. 1901 Fishy flavored butter: The cause and remedy. The Agr. Gaz. N. S. Wales, xii, 341.
- (3) REAKES, C. J., CUDDIE, D., AND REID, H. A. 1912 Jour. New Zeal. Dept. Agr., iv, no. 1, pp. 1-6.
- (4) HARRISON, J. 1901 Fishy flavor in butter. N. Y. Produce Review and Am. Creamery, xiii, 20.
- (5) ROGERS, L. A. 1909 Fishy flavor in butter. U. S. Dept. Agr. Bur. Animal Ind. Cir. 146.
- (6) HUNZIKER, O. F. 1915 Report of Department of Dairy Husbandry. 28th Ann. Rpt. Purdue Univ. Exp. Sta., p. 39.
- (7) HAMMER, B. W. 1917 Fishiness in evaporated milk. Iowa Agr. Exp. Sta. Research Bul. 38.

## THE ELEVENTH ANNUAL STUDENTS' NATIONAL CONTEST IN JUDGING DAIRY CATTLE

HELMER RABILD

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The eleventh annual Students' National Contest in Judging Dairy Cattle was held at the National Dairy Show in Chicago, Illinois, October 6, 1919. The contest was open to teams of three men each, from all agricultural colleges. Teams from fifteen colleges participated in the contest. The states of Wisconsin, Indiana and South Carolina were represented for the first time.

Twelve animals of each of four dairy breeds were judged, namely, four bulls, four cows and four heifers; and the students were required to place these animals in their proper order of excellence. This placing was then compared with the official contest placing, which was determined by a committee consisting of coaches of the various teams.

*DeLaval Scholarship (\$400).* The DeLaval scholarship offered by the DeLaval Separator Company was won by Mr. Roy W. Ingham of the University of Nebraska for doing the best work in judging all breeds.

*Holstein-Friesian Scholarship (\$400).* The Holstein-Friesian scholarship offered by the Holstein-Friesian Association of America to the student standing highest in the judging of Holsteins was won by Mr. E. H. Taylor of the Massachusetts Agricultural College.

*Jersey Scholarship (\$400).* The Jersey scholarship offered by the American Jersey Cattle Club to the student doing the best work in judging Jerseys was won by Mr. L. A. Jessup of Purdue University, Indiana.

*The National Dairy Association Medals.* The National Dairy Association awarded gold medals to the five highest, or sweepstakes, winners judging all breeds, as follows:

1. Roy W. Ingham.....University of Nebraska
2. David Gilkerson.....South Dakota Agricultural College
3. E. E. Gottman.....Kansas State Agricultural College
4. H. C. Cole.....University of Wisconsin
5. W. F. Dove.....Iowa State College

*Official judges*

Minnesota.....	J. C. Cort
Wisconsin.....	R. S. Hulce
Iowa.....	Carl Weaver
Nebraska.....	B. H. Thompson
Kansas.....	H. W. Cave
Missouri.....	W. W. Swett
Indiana.....	L. H. Fairchild
New York.....	H. H. Wing
South Carolina .....	W. W. Fitzpatrick
New Hampshire .....	J. M. Fuller
Massachusetts.....	J. C. McNutt
Washington ..	E. G. Woodward
South Dakota.....	H. Jones
Maryland.....	D. Meade
Ohio.....	C. T. Conklin

R. S. Hulce      H. Jones

*Winners of trophies and scholarships*

Ayrshire Trophy.....	New York State College
Guernsey Trophy. ....	Iowa State College
Holstein Trophy.....	Kansas State Agricultural College
Jersey Trophy .....	University of Missouri
Hoard's Dairyman Sweepstakes Trophy,	
	Kansas State Agricultural College
National Dairy Show Sweepstakes Trophy,	
	Kansas State Agricultural College
J. B. Ford Company Cup ..	South Dakota State College
Holstein Scholarship, E. H. Taylor, Massachusetts Agricultural College	
Jersey Scholarship.....	L. A. Jessup, Purdue University, Indiana
DeLaval Scholarship.....	Roy Ingham, University of Nebraska

*Report of teams in judging all breeds*

Basis of award of { National Dairy Association cup  
Hoard's Dairyman cup

## 2. J. B. Ford cup

RANK	TEAM	SCORE
1	Kansas State Agricultural College.....	210
2	South Dakota State College.....	232
3	Iowa State College.....	242 H. C.
4	University of Minnesota.....	242 L. C.
5	University of Nebraska.....	246
6	Washington State College.....	249
7	Ohio State University.....	268
8	Clemson Agricultural College.....	278
9	Purdue University.....	279
10	New York State College of Agriculture.....	282
11	University of Wisconsin.....	305
12	Massachusetts Agricultural College.....	316
13	Maryland State College.....	322
14	University of Missouri.....	331
15	New Hampshire College.....	337

*Report of teams in judging Ayrshires*

## Ayrshire cup

1	New York State College .....	31
2	University of Minnesota.....	36
3	Washington State College.....	49
4	Massachusetts Agricultural College.....	55
5	Ohio State University.....	61
6	New Hampshire College.....	62
7	Kansas State Agricultural College.....	65
8	South Dakota State College.....	69
9	Clemson Agricultural College.....	72
10	University of Nebraska.....	75
11	University of Wisconsin.....	78
12	Purdue University.....	87
13	Maryland State College.....	90
14	Iowa State College.....	95
15	University of Missouri.....	111

*Report of teams in judging Guernseys*

## Basis of award of Guernsey cup

RANK	TEAM	SCORE
1	Iowa State College.....	37
2	Washington State College.....	41
3	Purdue University.....	45
4	University of Minnesota.....	46
5	South Dakota State College.....	47
6	University of Wisconsin.....	54
7	Clemson Agricultural College .....	58
8	New York State College.....	59
9	University of Nebraska.....	65
10	Maryland State College.....	67
11	Kansas State Agricultural College.....	77
12	Ohio State University.....	100
13	University of Missouri.....	103
14	New Hampshire College.....	108
15	Massachusetts Agricultural College.....	128

*Report of teams in judging Holsteins*

## Basis of award of Holstein-Friesian cup

1	Kansas State Agricultural College .....	20
2	Iowa State College.....	52
3	Ohio State University.....	55
4	South Dakota State College .....	57
5	University of Nebraska.....	58
6	Maryland State College.....	60
7	New Hampshire College.....	64
8	Massachusetts Agricultural College.....	72
9	New York State Agricultural College.....	74
10	Clemson Agricultural College .....	77
11	University of Missouri.....	84
12	Purdue University.....	87
13	Wisconsin University.....	88 H. C.
14	Washington State College.....	88 L. C.
15	University of Minnesota .....	99



*Report of teams in judging Jerseys*  
Jersey cup

RANK	TEAM	SCORE
1	University of Missouri.....	33
2	University of Nebraska.....	48 H. C.
3	Kansas State Agricultural College.....	48 L. C.
4	Ohio State University.....	52
5	Iowa State College.....	58
6	Purdue University.....	60
7	Massachusetts Agricultural College.....	61 H. C.
8	University of Minnesota.....	61 L. C.
9	South Dakota State College.....	62
10	Clemson Agricultural College .....	70
11	Washington State College.....	71
12	University of Wisconsin.....	85
13	New Hampshire College.....	103
14	Maryland State College.....	105
15	New York State College of Agriculture.....	118

*Report of individuals in judging all breeds*

## DeLaval separator scholarship and five gold medals

RANK	ENTRY NUM- BER	NAME	COLLEGE	SCORE
1	6	Roy W. Ingham	University of Nebraska	32
2	44	David Gilkerson	South Dakota State College	44
3	28	E. E. Gottman	Kansas State Agricultural College	45
4	8	H. C. Cole	University of Wisconsin	59 H. C.
5	34	W. F. Dove	Iowa State College	59 L. C.
6	32	J. C. Knott	Washington State College	61
7	24	H. M. Kaldahl	University of Minnesota	64
8	20	L. A. Jessup	Purdue University	65
9	30	Raymond Campbell	Kansas State Agricultural College	69
10	37	E. H. Taylor	Massachusetts Agricultural College	71
11	40	W. M. McVey	Ohio State University	74
12	4	Don Q. Douglas	University of Nebraska	75
13	14	J. W. Beiermeister	New York State College of Agriculture	76
14	10	F. M. Allen	Clemson Agricultural College	77
15	31	Chas. Hansen	Washington State College	82
16	45	H. E. Urton	South Dakota State College	83
17	11	Thos. H. Burgess	Clemson Agricultural College	84
18	2	J. A. Gray	Maryland State College	86 H. C.
19	23	C. B. Finley	University of Minnesota	86 2d H. C.
20	42	E. D. Lenhart	Ohio State University	86 L. C.
21	35	L. A. Bent	Iowa State College	87
22	22	R. H. Steidl	University of Minnesota	91
23	7	Chas. B. Drewry	University of Wisconsin	93
24	29	G. C. Anderson	Kansas State Agricultural College	95
25	36	H. B. Davel	Iowa State College	96
26	16	H. J. Harling	New Hampshire College	101 H. C.
27	5	E. T. Itschner	University of Missouri	101 2d H. C.
28	15	Martin G. Beck	New York State College of Agriculture	101 L. C.
29	26	W. J. Keegan	University of Missouri	104
30	43	Bernard Iverson	South Dakota State College	105 H. C.
31	13	Francis J. Oates	New York State College of Agriculture	105 2d H. C.
32	19	J. M. Kirkpatrick	Purdue University	105 L. C.
33	33	H. O. Lisle	Washington State College	106
34	41	J. L. Hirsch	Ohio State University	108

*Report of individuals in judging all breed—continued*

RANK	ENTRY NUM- BER	NAME	COLLEGE	SCORE
35	21	B. E. Sellers	Purdue University	109
36	3	W. C. Snarr	Maryland State College	114 H. C.
37	17	A. B. Brown	New Hampshire College	114 L. C.
38	12	S. A. McGee	Clemson Agricultural College	117 H. C.
39	39	E. E. Harvey	Massachusetts Agricultural College	117 L. C.
40	18	R. J. Young	New Hampshire College	122 H. C.
41	1	John R. Drawbaugh	Maryland State College	122 L. C.
42	27	John Crosser	University of Missouri	126
43	38	A. C. Williams	Massachusetts Agricultural College	128
44	5	P. B. Campbell	University of Nebraska	139
45	9	Sidney P. Murat	University of Wisconsin	153

*Report of individuals in judging Ayrshires*

1	23	C. B. Finley	University of Minnesota	22
2	30	Raymond Campbell	Kansas State Agricultural College	23
3	6	Roy W. Ingham	University of Nebraska	28 H. C.
4	44	David Gilkerson	South Dakota State College	28 L. C.
5	14	J. W. Beiermeister	New York State College of Agriculture	50
6	7	Charles B. Drewry	University of Wisconsin	65
7	3	W. C. Snarr	Maryland State College	70 H. C.
8	13	Francis J. Oates	New York State College of Agriculture	70 L. C.
9	22	R. H. Steidl	University of Minnesota	75 H. C.
10	39	E. E. Harvey	Massachusetts Agricultural College	75 L. C.
11	16	H. J. Harling	New Hampshire College	76
12	19	J. M. Kirkpatrick	Purdue University	79
13	11	Thomas H. Burgess	Clemson Agricultural College	83 H. C.
14	31	Chas. Hansen	Washington State College	83 L. C.
15	41	J. L. Hirsch	Ohio State University	84
16	33	H. O. Lisle	Washington State College	85
17	37	E. H. Taylor	Massachusetts Agricultural College	87
18	15	Martin G. Beck	New York State College of Agriculture	88
19	32	J. C. Knott	Washington State College	89

*Report of individuals in judging Ayrshires—continued*

RANK	ENTRY NUM- BER	NAME	COLLEGE	SCORE
20	28	E. E. Gottman	Kansas State Agricultural College	90
21	18	R. J. Young	New Hampshire College	93
22	40	W. M. McVey	Ohio State University	94
23	10	F. M. Allen	Clemson Agricultural College	97 H. C.
24	42	E. D. Lenhart	Ohio State University	97 L. C.
25	34	W. F. Dove	Iowa State College	99 H. C.
26	24	H. M. Kaldahl	University of Minnesota	99 L. C.
27	45	H. E. Urton	South Dakota State College	100
28	38	A. C. Williams	Massachusetts Agricultural College	103 H. C.
29	35	L. A. Bent	Iowa State College	103 L. C.
30	17	A. B. Brown	New Hampshire College	104 H. C.
31	26	W. J. Keegan	University of Missouri	104 L. C.
32	5	P. B. Campbell	University of Nebraska	105 H. C.
33	20	L. A. Jessup	Purdue University	105 L. C.
34	9	Sidney P. Murat	University of Wisconsin	109 H. C.
35	27	John Crosser	University of Missouri	109 L. C.
36	43	Bernard Iverson	South Dakota State College	111
37	12	S. A. McGee	Clemson Agricultural College	117
38	8	H. C. Cole	University of Wisconsin	120
39	2	J. A. Gray	Maryland State College	127 H. C.
40	4	Don Q. Douglas	University of Nebraska	127 L. C.
41	36	H. B. Davel	Iowa State College	132
42	21	B. F. Sellers	Purdue University	135
43	29	G. C. Anderson	Kansas State Agricultural College	188
44	21	John R. Drawbaugh	Maryland State College	190
45	25	E. T. Itschner	University of Missouri	213

*Report of individuals in judging Guernseys*

1	32	J. C. Knott	Washington State College	23
2	11	Thos. H. Burgess	Clemson Agricultural College	28
3	8	H. C. Cole	University of Wisconsin	40
4	20	L. A. Jessup	Purdue University	44
5	28	E. E. Gottman	Kansas State Agricultural College	45
6	44	David Gilkerson	South Dakota State College	54
7	24	H. M. Kaldahl	University of Minnesota	55
8	15	Martin G. Beck	New York State College of Agriculture	62

*Report of individuals in judging Guernseys—continued*

RANK	ENTRY NUM- BER	NAME	COLLEGE	SCORE
9	34	W. F. Dove	Iowa State College	65
10	2	J. A. Gray	Maryland State College	67 H. C.
11	6	Roy W. Ingham	University of Nebraska	67 L. C.
12	23	C. B. Finley	University of Minnesota	72
13	36	H. B. Davel	Iowa State College	77
14	31	Charles Hansen	Washington State College	79
15	35	L. A. Bent	Iowa State College	83
16	25	E. T. Itschner	University of Missouri	85
17	7	Chas. Drewry	University of Wisconsin	86
18	10	F. M. Allen	Clemson Agricultural College	87 H. C.
19	19	J. M. Kirkpatrick	Purdue University	87 L. C.
20	45	H. E. Urton	South Dakota State College	91 H. C.
21	43	Bernard Iverson	South Dakota State College	91 L. C.
22	21	B. F. Sellers	Purdue University	93
23	14	J. W. Beiermeister	New York State College of Agriculture	95
24	1	John R. Drawbaugh	Maryland State College	96
25	4	Don Q. Douglas	University of Nebraska	97 H. C.
26	33	H. O. Lisle	Washington State College	97 L. C.
27	22	R. H. Steidl	University of Minnesota	102 H. C.
28	13	Francis J. Oates	New York State College of Agriculture	102 L. C.
29	5	P. B. Campbell	University of Nebraska	105 H. C.
30	42	E. D. Lenhart	Ohio State University	105 L. C.
31	40	W. M. McVey	Ohio State University	108 H. C.
32	30	Raymond Campbell	Kansas State Agricultural College	108 2d H. C.
33	3	W. C. Snarr	Maryland State College	108 L. C.
34	9	Sidney P. Murat	University of Wisconsin	109
35	16	H. J. Harling	New Hampshire College	110 H. C.
36	17	A. B. Brown	New Hampshire College	110 L. C.
37	18	R. J. Young	New Hampshire College	116
38	12	S. A. McGee	Clemson Agricultural College	121
39	41	J. L. Hirsch	Ohio State University	122
40	29	G. C. Anderson	Kansas State Agricultural College	124
41	37	E. H. Taylor	Massachusetts Agricultural College	137
42	27	John Crosser	University of Missouri	156
43	39	E. E. Harvey	Massachusetts Agricultural College	157
44	38	A. C. Williams	Massachusetts Agricultural College	160
45	26	W. J. Keegan	University of Missouri	172

*Report of individuals in judging Holsteins*  
Holstein scholarship

RANK	ENTRY NUM- BER	NAME	COLLEGE	SCORE
1	37	E. H. Taylor	Massachusetts Agricultural College	37
2	28	E. E. Gottman	Kansas State Agricultural College	48
3	2	J. A. Gray	Maryland State College	49
4	29	G. C. Anderson	Kansas State Agricultural College	53
5	17	A. B. Brown	New Hampshire College	65
6	4	Don Q. Douglas	University of Nebraska	66 H. C.
7	36	H. B. Davel	Iowa State College	66 L. C.
8	14	J. W. Beiermeister	New York State College of Agriculture	67
9	8	H. C. Cole	University of Wisconsin	68
10	10	F. M. Allen	Clemson Agricultural College	71
11	44	David Gilkerson	South Dakota State College	75 H. C.
12	1	John R. Drawbaugh	Maryland State College	75 L. C.
13	6	Roy W. Ingham	University of Nebraska	79 H. C.
14	30	Raymond Campbell	Kansas State Agricultural College	79 L. C.
15	34	W. F. Dove	Iowa State College	80
16	42	E. D. Lenhart	Ohio State University	84 H. C.
17	21	B. F. Sellers	Purdue University	84 L. C.
18	40	W. M. McVey	Ohio State University	87 H. C.
19	18	R. J. Young	New Hampshire College	87 2d H. C.
20	24	H. M. Kaldahl	University of Minnesota	87 L. C.
21	41	J. L. Hirsch	Ohio State University	92
22	45	H. E. Urton	South Dakota State College	96
23	32	J. C. Knott	Washington State College	97
24	43	Bernard Iverson	South Dakota State College	97 L. C.
25	27	John Crosser	University of Missouri	99
26	26	W. J. Keegan	University of Missouri	101
27	20	L. A. Jessup	Purdue University	109
28	33	H. O. Lisle	Washington State College	113
29	38	A. C. Williams	Massachusetts Agricultural college	114
30	35	L. A. Bent	Iowa State College	115
31	11	Thomas H. Burgess	Clemson Agricultural College	119 H. C.
32	13	Francis J. Oates	New York State College of Agriculture	119 L. C.
33	25	E. T. Itschner	University of Missouri	124
34	15	Martin G. Beck	New York State College of Agriculture	125

*Report of individuals in judging Holsteins—continued*

RANK	ENTRY NUM- BER	NAME	COLLEGE	SCORE
35	22	R. H. Steidl	University of Minnesota	129
36	12	S. A. McGee	Clemson Agricultural College	133
37	31	Charles Hansen	Washington State College	142
38	7	Charles B. Drewry	University of Wisconsin	145 H. C.
39	5	P. B. Campbell	University of Nebraska	145 L. C.
40	16	H. J. Harling	New Hampshire College	146
41	9	Sidney P. Murat	University of Wisconsin	148
42	39	E. E. Harvey	Massachusetts Agricultural College	154
43	19	J. M. Kirkpatrick	Purdue University	156
44	23	C. B. Finley	University of Minnesota	168
45	3	W. C. Snarr	Maryland State College	175

*Report of individuals judging Jerseys*

## Jersey scholarship

1	20	L. A. Jessup	Purdue University	25
2	26	W. J. Keegan	University of Missouri	40
3	40	W. M. McVey	Ohio State University	41
4	4	Don Q. Douglas	University of Nebraska	47
5	6	Roy W. Ingham	University of Nebraska	49 H. C.
6	12	S. A. McGee	Clemson Agricultural College	49 L. C.
7	25	E. T. Itschner	University of Missouri	52
8	29	G. C. Anderson	Kansas State Agricultural College	53
9	8	H. C. Cole	University of Wisconsin	54 H. C.
10	34	W. F. Dove	Iowa State College	54 L. C.
11	24	H. M. Kaldahl	University of Minnesota	64
12	37	E. H. Taylor	Massachusetts Agricultural College	67
13	35	L. A. Bent	Iowa State College	73
14	45	H. E. Urton	South Dakota State College	77
15	16	H. J. Harling	New Hampshire College	79
16	42	E. D. Lenhart	Ohio State University	81
17	31	Chas. Hansen	Washington State College	84
18	32	J. C. Knott	Washington State College	85 H. C.
19	28	E. E. Gottman	Kansas State Agricultural College	85 L. C.
20	22	R. H. Steidl	University of Minnesota	87
21	30	Raymond Campbell	Kansas State Agricultural College	90

*Report of individuals judging Jerseys—continued*

RANK	ENTRY NUM- BER	NAME	COLLEGE	SCORE
22	39	E. E. Harvey	Massachusetts Agricultural College	91
23	44	David Gilkerson	South Dakota State College	95
24	27	John Crosser	University of Missouri	101 H. C.
25	43	Bernard Iverson	South Dakota State College	101 L. C.
26	10	F. M. Allen	Clemson Agricultural College	102
27	38	A. C. Williams	Massachusetts Agricultural College	104
28	21	B. F. Sellers	Purdue University	105
29	3	W. C. Snarr	Maryland State College	110 H. C.
30	23	C. B. Finley	University of Minnesota	110 L. C.
31	19	J. M. Kirkpatrick	Purdue University	114
32	7	Chas. B. Drewry	University of Wisconsin	117
33	41	J. L. Hirsch	Ohio State University	121
34	2	J. A. Gray	Maryland State College	124
35	36	H. B. Davel	Iowa State College	125
36	33	H. O. Lisle	Washington State College	130
37	13	Francis J. Oates	New York State College of Agriculture	142
38	11	Thomas H. Burgess	Clemson Agricultural College	153 H. C.
39	5	P. B. Campbell	University of Nebraska	153 L. C.
40	14	J. W. Beiermeister	New York State College of Agriculture	155
41	15	Martin G. Beck	New York State College of Agriculture	161
42	1	John R. Drawbaugh	Maryland State College	164
43	17	A. B. Brown	New Hampshire College	165
44	9	Sidney P. Murat	University of Wisconsin	184
45	18	R. J. Young	New Hampshire College	185



*Contestants' numbers by colleges*

NUMBER	NAME	COLLEGE
N 1	John R. Drawbaugh	Maryland State College
O 2	J. A. Gray	Maryland State College
P 3	W. C. Snarr	Maryland State College
Q 4	Don Q. Douglas	University of Nebraska
N 5	P. B. Campbell	University of Nebraska
O 6	Roy W. Ingham	University of Nebraska
P 7	Charles B. Drewry	University of Wisconsin
Q 8	H. C. Cole	University of Wisconsin
N 9	Sidney P. Murat	University of Wisconsin
O 10	F. M. Allen	Clemson Agricultural College
P 11	Thomas H. Burgess	Clemson Agricultural College
Q 12	S. A. McGee	Clemson Agricultural College
N 13	Francis J. Oates	New York State College of Agriculture
O 14	J. W. Beiermeister	New York State College of Agriculture
P 15	Martin G. Beck	New York State College of Agriculture
Q 16	H. J. Harling	New Hampshire College
N 17	A. B. Brown	New Hampshire College
O 18	R. J. Young	New Hampshire College
P 19	J. M. Kirkpatrick	Purdue University
Q 20	L. A. Jessup	Purdue University
N 21	B. F. Sellers	Purdue University
O 22	R. H. Steidl	University of Minnesota
P 23	C. B. Finley	University of Minnesota
Q 24	H. M. Kaldahl	University of Minnesota
N 25	E. T. Itschner	University of Missouri
O 26	W. J. Keegan	University of Missouri
P 27	John Crosser	University of Missouri
Q 28	E. E. Gottman	Kansas State Agricultural College
N 29	G. C. Anderson	Kansas State Agricultural College
O 30	Raymond Campbell	Kansas State Agricultural College
P 31	Charles Hansen	Washington State College
Q 32	J. C. Knott	Washington State College
N 33	H. O. Lisle	Washington State College

*Contestants' numbers by colleges—continued*

NUMBER	NAME	COLLEGE
O 34	W. F. Dove	Iowa State College
P 35	L. A. Bent	Iowa State College
Q 36	H. B. Davel	Iowa State College
N 37	E. H. Taylor	Massachusetts Agricultural College
O 38	A. C. Williams	Massachusetts Agricultural College
P 39	E. E. Harvey	Massachusetts Agricultural College
Q 40	W. M. McVey	Ohio State University
N 41	J. L. Hirsch	Ohio State University
O 42	E. D. Lenhart	Ohio State University
P 43	Bernard Iverson	South Dakota State College
Q 44	David Gilkerson	South Dakota State College
N 45	H. E. Urton	South Dakota State College

# A PRELIMINARY REPORT ON THE STUDY OF THE TEMPERATURES AT WHICH MILK OF DIFFERENT PER CENTS OF ACIDITY WILL COAGULATE

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According to Hammersten<sup>1</sup> "perfectly fresh amphoteric milk does not coagulate on boiling but forms a pellicle consisting of casein and lime-salts which rapidly reforms after being removed. Even after passing a current of carbon dioxide through the fresh milk it does not coagulate on boiling. In proportion as the formation of lactic acid advances this behavior changes and soon a stage is reached when the milk, which has previously had carbonic acid passed through it, coagulates on boiling. At a second stage it coagulates alone on heating, thus it coagulates by passing carbon dioxide alone without boiling; and lastly when the formation of lactic acid is sufficient, it coagulates spontaneously at the ordinary temperature forming a solid mass. It may also happen especially in the warmth, that the casein clot contracts and a yellowish or yellowish green acid liquid (acid whey) separates."

According to Van Slyke, "when milk is treated with acid or acid salts the casein is precipitated as a heavy, white solid in more or less flocculent form, depending on conditions of treatment. When milk sours in the ordinary way the lactic acid that is formed produces the same effect as the addition of any dilute acid; the precipitation or curdling occurs at ordinary temperatures, when the acidity reaches 0.6 to 0.7 per cent expressed as lactic acid. When dilute artificial lactic acid is added directly to fresh milk, precipitation takes place at ordinary room temperature when the acidity reaches 0.57 per cent. Increase of temperature enables a smaller amount of acid to precipitate casein; for example, milk curdling at room tempera-

<sup>1</sup> A Text Book of Physiological Chemistry. Hammerstein and Mandel. From Allen's Commercial Organic Analysis, vol. viii, p. 121.

ture with 0.55 per cent of added lactic acid, precipitates with 0.35 per cent at boiling temperature, according to work done in the laboratory of the writer." The indicator used in determining milk acidity is phenolphthalein. Litmus cannot be used because of the amphoteric reaction of fresh milk. Hörner gives the following method of determining the acidity of milk.<sup>2</sup> Ten cubic centimeters of milk are diluted with 20 cc. of water a few drops of a dilute alcoholic solution of phenolphthalein added and the titration made with  $\frac{N}{10}$  alkali. Hörner proposes that the number of cubic centimeter required should be multiplied by 10 and the result termed the "degree of acidity." Fresh normal milk will show figures ranging from 16 to 18. When the degree of acidity is 23 or more, the sample will coagulate on heating.

Owing to the fact that modern methods of handling milk and its products frequently involves heating to a temperature of 145°F. or higher, it seemed desirable to secure further information regarding the relation between the percentage of acidity, the temperature and coagulation. For this purpose the following experiments were performed.

#### PRELIMINARY WORK

Eighteen grams of milk were titrated with  $\frac{N}{10}$  alkali solution using phenolphthalein as an indicator, the purpose being to determine the "degree" or per cent acidity of the milk. To another 18 gram sample of milk was added 100 cc. of distilled water. A solution of  $\frac{N}{10}$  lactic acid was then added until the curd was precipitated or the milk coagulated and the temperature was noted.

To a third sample was added 100 cc. of distilled water and a smaller volume of acid. The mixture of milk, water and acid was then heated until the milk coagulated and the temperature noted. This was continued for several per cents of acid and several temperatures. The method may be illustrated as follows: 18 gram milk + 100 cc. distilled water + 48 cc. of  $\frac{N}{10}$  lactic acid precipitated at 60°F.

<sup>2</sup> Chem. Zeit. 1892, 16, 1479, 1519.

Another method was to heat the mixture of 18 grams of milk and 100 cc. of distilled water to a given temperature and then add the  $\frac{N}{10}$  lactic acid solution until the milk curdled. In these experiments it was found that the milk when heated before adding the acid curdled at a lower temperature than was the case when the acid was first added and the mixture heated. The only explanation for this is that the acid on coming in contact with the warm milk may coagulate the part of the milk with which it comes in contact and the coagulation may not be uniform. In the other case when the acid is added to the milk before heating, the acid is evenly distributed through the milk and when the critical temperature is reached the entire mass of milk will coagulate. Later in the work it was found best to take 100 cc. portions of milk after the acid test had been made and add a certain amount of the  $\frac{N}{10}$  lactic acid. The mixture of milk and acid was then heated in a steam bath until the milk coagulated and the temperature was noted. The amount of acid varied from the amount required to coagulate the milk at room temperature to 180°F. Usually the difference in amount added each time was about 5 cc.

Over 200 samples of milk have been treated by the above methods and the figures given below are indicative of the results obtained.

	AMOUNT OF MILK	ACID	TEMPERATURE
<i>Experiment I</i>			
	cc.	per cent	°F.
Skim milk; 0.145 per cent acid . . . . .	100	0.580	70
	100	0.480	104
	100	0.430	145
	100	0.390	150
	100	0.340	155
	100	0.280	170
	100	0.250	185

*Experiment II*

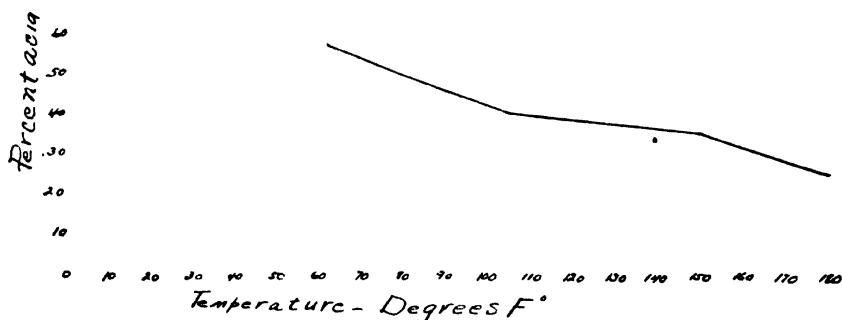
Skim milk; 0.14 per cent acid	100	0.570	68
	100	0.54	66

	AMOUNT OF MILK	ACID	TEMPERATURE
<i>Experiment III</i>			
Skim milk; 0.23 <sup>5</sup> per cent acid.....	100	0.520	74
<i>Experiment IV</i>			
Whole milk; 0.15 per cent acid .....	100	0.550	66
	100	0.410	105
	100	0.367	150
	100	0.320	157
<i>Experiment V</i>			
Skim milk; 0.17 per cent acid.....	100	0.520	74
	100	0.470	85
	100	0.430	125
	100	0.390	140
	100	0.340	157
	100	0.300	180
	100	0.260	195
<i>Experiment VI</i>			
Pasteurized milk (24 hours old), 0.18 per cent acid .....	100	0.480	67
	100	0.440	82
	100	0.398	100
	100	0.350	155
	100	0.310	165
	100	0.269	182
	100	0.220	200
<i>Experiment VII</i>			
Pasteurized milk (24 hours old); 0.15 per cent acid .....	cc.	per cent	°F.
	100	0.560	66
	100	0.500	85
	100	0.480	83
	100	0.450	95
	100	0.410	96
	100	0.400	104
	100	0.390	110
	100	0.370	140
	100	0.360	150
	100	0.320	160

	AMOUNT OF MILK	ACID	TEMPERATURE
<i>Experiment VIII</i>			
Whole milk; 0.14 per cent acid.....	100	0.530	73
	100	0.480	87
	100	0.440	110
	100	0.400	110
	100	0.350	147
	100	0.310	162
	100	0.270	175
<i>Experiment IX</i>			
Milk (24 hours old).....	100	0.500	76
	100	0.450	88
	100	0.400	100
	100	0.360	105
	100	0.340	153
	100	0.330	158
<i>Experiment X</i>			
Whole milk; 0.15 per cent acid.....	100	0.540	68
	100	0.495	72
	100	0.466	88
	100	0.440	96
	100	0.420	105
	100	0.397	150
	100	0.375	155
	100	0.330	160
	100	0.285	173
<i>Experiment XI</i>			
Whole milk; 0.40 per cent acid.....	100	0.527	67
	100	0.490	70
	100	0.472	74
	100	0.445	86
	100	0.427	88
	100	0.409	100
	100	0.400	110

These experiments show that milk containing 0.57 per cent acid (in terms of lactic acid) will, on the average, precipitate at a temperature between 60° to 65°F. Milk containing 0.50 per cent acid will curdle at 75° to 80°F., 0.40 per cent at 100° to

110°F., 0.35 per cent at about 150°F. and 0.25 per cent acid in milk will not cause coagulation until heated to 180°F. As shown in the curve, the small drop in acidity between 0.40 to 0.35 per cent makes a greater range of temperature than between any other two points of acidity studied. As shown in the experiments, a decrease of 0.05 per cent acid at this particular stage requires nearly a 50°F. range in temperature to produce coagulation as 0.40 per cent acid in milk will curdle at about 100°F. while 0.35 per cent acid in milk will not produce curdling until heated to at least 150°F.



CURVE SHOWING THE TEMPERATURE AT WHICH MILK OF DIFFERENT PER CENTS OF ACID WILL COAGULATE

The author believes that, when the milk is heated to about 100°F., a chemical change may take place in the calcium salts found in milk and in some way cause the casein to be held in suspension.

The addition of di-sodium phosphate will retard the precipitation of the curd in milk even though a high percentage of lactic acid is present. As an illustration, a sample of milk showing 0.60 per cent acid curdled when heated to 60°F. Another 18 gram sample of this milk was taken to which was added 20 cc. of 1 per cent solution of di-sodium phosphate ( $\text{Na}_2\text{HPO}_4$ ) and this sample of milk did not precipitate when heated to 175°F.

The addition of mono-calcium phosphate  $\text{CaH}_4(\text{PO}_4)_2$  has an entirely different effect than the sodium phosphate. The former will hasten the coagulation of milk as illustrated in the following



experiment. Twenty cubic centimeters of skim milk containing 0.18 per cent acid was used in the experiment. To this was added 44 cc. of a 1 per cent solution of mono-calcium phosphate ( $\text{CaH}_4(\text{PO}_4)_2$ ) and the sample curdled at 70°F. The result of these experiments would tend to show that the break in the curve, which is formed with the different per cents of acid and different temperatures may be due to the phosphates in milk.

Further study is being made as to the exact cause of the irregular curve produced from the above experiments.

## A NOTE ON THE ACIDITY OF FRESH MILK

T. J. McINERNEY

*Department of Dairy Industry, Cornell University, Ithaca, New York*

When milk is first drawn from the cow's udder it gives what is known as an amphoteric reaction, that is it turns red litmus paper blue and blue litmus paper red. In spite of this fact fresh milk is rarely alkaline and is always acid toward phenolphthalein as an indicator.

Dairy chemists mention two kinds of acidity in milk; apparent acidity and real acidity. The real acidity of milk is due to lactic acid. This is never found in milk when it is first drawn from the udder. It is produced by the action of the lactic acid organisms on the milk sugar.

The so-called apparent acidity of milk is what gives fresh milk its acid reaction. It is now generally believed to be due to the presence of the carbon dioxide, acid phosphates and casein, all of which are found in fresh milk and which have an acid.

About a year ago the writer tested two samples of milk that were brought to this department. This milk had been rejected at the local condensary because of high acidity. Both samples showed 0.21 per cent acidity when tested by the usual alkali solution. The writer was then requested to visit the farm on which this milk was produced and test the milk from each individual cow. Samples were taken and tested for acidity immediately, and the remainder taken to the laboratory from which chemical analyses were made for total solids, fat, solids not fat and ash. The results are as follows:

COW NUMBER	ACIDITY	TOTAL SOLIDS	FAT	SOLIDS NOT FAT	ASH
1	0.202	15.83	6.0	9.83	0.907
2	0.185	15.68	6.5	9.18	0.805
3	0.175	16.91	7.5	9.41	0.775
4	0.205	15.29	5.9	9.39	0.841
5	0.202	15.55	5.9	9.65	0.733
Herd	0.190	15.78	6.3	9.48	0.820

These were all grade Jersey cows. From the tests made of the milk immediately after milking, it would indicate that nearly all the cows were giving milk showing a high per cent of acidity as shown by the alkali test. All these samples show a very high percentage of total solids, fat and solids not fat. The per cent of ash in all cases is higher than the average of normal milk, and in one or two cases the percentage of ash is exceedingly high. These facts would tend to show the reason for the high apparent acidity in the fresh milk.

To make a more complete study of the acidity in fresh milk, it was decided to determine the acidity of the milk of the different cows of the University herd. This herd was selected because five different breeds of cattle were represented and the age of the cow and the period of lactation of each individual animal was known. The breeds that were available for study were the Holstein, Shorthorn, Ayrshire, Guernsey and Jersey. There were 15 Holstein, 10 Jersey, 6 Guernsey, 5 Ayrshire, and 5 Shorthorns, making a total of 42 tests in all, or one from each cow. The samples were obtained immediately after the night milking and tests for acidity were made in about one-half hour after the samples were drawn.

The average per cent of acidity as shown by the alkali test for each breed in the herd and the highest and lowest per cents of acidity found in the milk of each breed are shown in the following table:

BREED	AVERAGE PER CENT ACID	HIGHEST PER CENT FOUND	LOWEST PER CENT FOUND
Holstein.....	0.136	0.180	0.100
Jersey.....	0.162	0.220	0.130
Guernsey.....	0.139	0.160	0.120
Ayrshire.....	0.123	0.150	0.105
Short Horn.....	0.180	0.200	0.165

The results from the data given above may be summarized as follows:

1. The per cent of apparent acid found in the fresh milk varied from 0.10 to 0.22 per cent.

2. There appears to be no relation between the period of lactation and the percentage of apparent acid in the milk.

3. There appears to be no relation between the age of the cow and the percentage of apparent acid found in the milk.

4. The kinds of feed had no effect on the percentage of acid found in the milk.

5. Fresh milk which showed a high percentage of solids not fat and a high percentage of ash showed a high percentage of apparent acidity.

6. Fresh milk comparatively low in solids not fat and in ash showed a lower percentage of apparent acidity.

## OPEN FORUM

### A SCORE CARD FOR CITY ICE CREAM PLANTS<sup>1</sup>—F. W. FABIAN

The output of the ice cream industry has greatly increased in the last decade and likewise its commercial value. We now recognize it as one of the many American industries. But while the output and value have greatly increased, the sanitary measures have not kept pace with the industry. In some places possibly they have kept pace and in others they have not. One thing is certain that on the whole the ice cream industry has not received as much attention from a sanitary point of view as some of its sister industries. For example, in most cities, we do not find any bacteriological standard for ice cream or the constituents that go into its make up. We do not have a score card for city ice cream plants as we do for city milk plants. Cities in which there is a system of country and city milk inspection, we find no provision for inspection of ice cream plants or the farms supplying the cream and milk for ice cream. Many cities realizing the great need of sanitary inspection are taking steps to provide for this inspection.

It was while working for the Detroit Board of Health at Detroit, Michigan, in the summer of 1917 in the capacity of a sanitary inspector for ice cream plants, that my attention was called to the need of two things: First, regular sanitary inspection of all places large or small manufacturing ice cream, and second, a systematic way of recording the inspection.

Inquiry was then made of the Bureau of Animal Industry as to whether they knew of a score card for ice cream plants and they replied that they had never issued such a score card and knew of none, unless, the Louisiana State Board of Health issued one. Upon writing to them and having them forward a copy of their score card it was found that it was not a score card at all but simply a memorandum card upon which the inspector made general notations of the sanitary condition of the place, but it was a step in the right direction, however, and a great improvement upon no score card.

<sup>1</sup> Through an error, part of the score card was omitted from the article which was published in the November issue. In justice to the author, the entire article is reprinted in this issue.—EDITOR.

I am sure that no one familiar with the situation would doubt the need of regular sanitary inspection by a competent inspector. Here we have a plant manufacturing a product which is easily contaminated, and quite frequently is being handled by employees who are ignorant of the dangers that they may introduce by their ignorance and carelessness.

Now if this product were fed to cattle or utilized in any other way than it is, our problem would cease to exist; but since it is included in the human diet and occupies such a prominent place, it certainly needs careful sanitary supervision and inspection. Ice cream is not only used quite extensively in the normal healthy human diet, but is often recommended by physicians for the young, the old and convalescents alike. The same physician who would hesitate to recommend anything but certified milk (or some milk equally dependable) with a bacteria count of 10,000 or less, does not hesitate to recommend ice cream with no bacteria standard whatever, but which usually ranges from 10,000 to 1,000,000,000 bacteria per cubic centimeter.

The fact that ice cream is frozen seems to cover a multitude of sins in the layman's mind. Just as the farmer and most other people believe that straining and clarifying make dirty milk clean, likewise people believe that freezing kills all bacteria. All of these facts argue very strongly for regular sanitary inspection.

The duties of an inspector of ice cream plants, as in the case of any other sanitary inspector, are many. If he educates one man one way, advises another in another way, and assists the others in other ways, the sum total of his labors are varied and his efforts result in confusion. The best way to help all and secure uniform results is by the score card. In this way there is an outline to guide him and he can accord to all the same help and treatment.

The score card for ice cream plants as it is arranged here follows in general outline the score cards for city milk plants as approved by the United States Department of Agriculture, but is arranged to meet the needs of city ice cream plants. The ice cream plant is scored on the basis of 100 per cent as perfect, 40 per cent for equipment, and 60 per cent for methods.

The sanitary location of the building is the first consideration. Next comes the arrangement of the building providing a separate room for each major operation. The construction of the rooms and other sanitary considerations as drainage, light, pure air, screens, etc., are next taken up. The apparatus necessary for keeping the machinery and

utensils clean and sanitary, also the apparatus for handling ice cream in a sanitary way, together with the condition of the machinery are given consideration. Laboratory and equipment are also taken into account as well as the water supply.

The sanitary methods are considered from the viewpoint of the cleanliness of the building, such as floors, walls etc. The cleanliness and protection from contamination of the apparatus used in making, handling and storing the ice cream both before and after making are given consideration. The way in which the constituents used in making the ice cream are received, protected and cared for before they are used for ice cream is given a very prominent place. The score card makes it possible for the man who goes to the trouble and expense of installing a pasteurizer to get a better score than the man who does not. There are many arguments pro and con in regard to pasteurization of the constituents of ice cream but there certainly is no question as to the ultimate outcome of the matter. The time is not far distant when all cities shall require the constituents of ice cream to be pasteurized. A place is given to the wrapping of brick ice cream which is often wrapped in a very unsanitary way. Storage and protection during delivery are likewise given a place. The bacteriological analysis of both raw materials and the finished product as well as the inspection of the dairies supplying cream are included under the head of inspection. This should help to produce a purer product. Then under "miscellaneous" comes the cleanliness of attendants and provision for medical inspection, thus helping to decrease the possibility of pathogenic organisms in the final product after all other precautions have been taken. Finally, comes the sanitary outfit to carry a sanitary product to its destination, provided all the other provisions in the score card have been lived up to.

The score card follows:

BOARD OF HEALTH  
SANITARY INSPECTION OF CITY ICE CREAM PLANTS  
SCORE CARD

Owner or manager.....  
 Street and No.....  
 City.....State.....  
 Trade name .....

Number of wagons.....	Gallons sold daily {	Bulk ice cream.....
		Brick ice cream.....

Permit or License No.....  
 Date of inspection.....192  
 Remarks...  
 .....  
 .....  
 .....  
 .....  
 .....  
 .....Inspector.

Arranged by F. W. Fabian, Michigan Agricultural College, Department of Bacteriology and Hygiene, East Lansing, Michigan.



EQUIPMENT	SCORE	
	Perfect	Allowed
Building:		
Location:		
Sanitary surroundings.....	2	.....
Arrangement.....	9	
Separate receiving room..... 1		
Separate freezing room..... 2		
Separate mixing room..... 2		
Separate wash room..... 1		
Separate sales room..... 1		
Separate boiler room..... 1		
Separate refrigerator room..... 1		
Construction.....	12	.....
Floors, tight, sound, cleanable..... 2		
Walls, tight, smooth, cleanable..... 1		
Ceilings, smooth, tight, cleanable..... 1		
Drainage.....	2	
Floors..... 1		
Sewer or septic tank..... 1		
Provision for light..... 2		
(10 per cent of floor space).		
Provision for pure air..... 2		
Screens (windows, doors)..... 1		
Minimum of shafting, pulleys, hangers, exposed pipes, etc..... 1		
Apparatus.....	13	.....
Boiler..... 2		
(Water heater, 1)		
Appliances for cleansing utensils and cans..... 1		
Sterilizers for cans, etc..... 2		
Wrapping machine (brick ice cream)..... 2		
Clean table for hand wrapping..... 1		
Wash bowl, soap, and towel in handling room.. 1		
Condition..... 4		
Ice cream freezing and mixing machinery..... 2		
Pipes, couplings, and pumps..... 1		
Cans and containers..... 1		
Laboratory and equipment.....	2	.....
Water supply.....	2	.....
Clean and fresh..... 1		
Convenient and abundant..... 1		
Total.....	40	.....

METHODS	SCORE	
	Perfect	Allowed
Building.....	14	.....
Cleanliness:		
Floors.....	3	
Walls.....	2	
Ceilings.....	2	
Doors and windows.....	1	
Shafting, pulleys, pipes, etc.....	1	
Freedom from odors.....	2	
Freedom from flies.....	3	
Apparatus.....	7	.....
Cleanliness:		
Thoroughly washed and rinsed.....	3	
Machinery handling mix.....	2	
Pipes, cans, etc.....	1	
Sterilized with live steam.....	3	
Machinery handling mix.....	2	
Pipes, cans, etc.....	1	
Protected from contamination.....	1	
Ice cream containers.....	7	.....
Thoroughly washed and rinsed.....	3	
Sterilized with steam 15 minutes.....	3	
Inverted in clean place.....	1	
Handling cream, milk, etc.....	21	.....
Received below 50°F.....	3	
(50°F. to 55°F. 2)		
(55°F. to 60°F. 1)		
Freedom from undue exposure to air.....	1	
Cooling.....	4	
Promptness.....	2	
Below 45°F.....	2	
Pasteurization of raw materials at 145°F. for 30 min. and promptly cooled to 45°F. or below..	4	
Bricks wrapped by machine.....	2	
(Bricks wrapped by hand, 1).....		
Ice cream container protected by cover.....	1	
Storage of ice cream at 0°F.....	4	
(0°F. to 5°F. 3; 5°F. to 10°F. 1)		
Protection during delivery.....	2	
(Iced during entire year)		
Inspection.....	6	.....
Bacteriological work.....	3	
(Raw materials, 1, Finished product 2)		
Inspection of dairies supplying cream.....	3	
(2 times a year, 2; once a year, 1)		
Miscellaneous.....	5	.....
Cleanliness of attendants.....	2	
(Personal cleanliness, 1; clean, washable clothing, 1).		
Medical inspection of employees handling products.....	1	
Cleanliness of delivery outfit.....	2	
Total.....	60	.....

## COMMITTEE APPOINTMENTS FOR THE AMERICAN DAIRY SCIENCE ASSOCIATION FOR 1920\*

### *Dairy Farm Score Card*

ERNEST KELLY, <i>Chairman</i>	W. A. STOCKING
C. B. LANE	I. C. WELD
H. A. HARDING	P. M. BRANDT

### *Milk Quality*

H. A. HARDING, <i>Chairman</i>	R. S. BREED
W. A. STOCKING	E. G. HASTINGS
J. D. BREW	F. RASMUSSEN

### *Bacteriological Methods for Market Milk*

R. S. BREED, <i>Chairman</i>	E. G. HASTINGS
L. A. ROGERS	B. W. HAMMER
J. D. BREW	

### *Relation to Breed Associations*

C. H. ECKLES, <i>Chairman</i>	E. G. WOODWARD
H. H. WING	W. W. YAPP
ROY T. HARRIS	G. C. WHITE
H. H. KILDEE	

### *Methods Conducting Student Dairy Cattle Judging Contest*

HELMER RABILD, <i>Chairman</i>	E. L. ANTHONY
H. H. WING	H. H. KILDEE
WILLIAM REGAN	J. B. FITCH
W. W. SWEET	

### *Official Methods for Testing Milk and Cream for Butter Fat*

O. F. HUNZIKER, <i>Chairman</i>	FRED RASMUSSEN
F. W. BOUSKA	H. C. TROY
L. A. ROGERS	

### *Legal Standards for Butter*

E. S. GUTHRIE, <i>Chairman</i>	G. H. BENKENDORF
S. C. THOMPSON	C. L. ROADHOUSE
F. W. BOUSKA	

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\* This is a complete list of committee appointments as just received from President Mortimer.—EDITOR.

*Statistics on Production and Marketing of Dairy Products*ROY C. POTTS, *Chairman*

S. C. THOMPSON

O. F. HUNZIKER

C. E. LEE

L. M. DAVIS

*Graduate Instruction in Dairying*W. A. STOCKING, *Chairman*

H. A. HARDING

R. A. PEARSON

C. H. ECKLES

C. W. LARSON

E. G. HASTINGS

*Courses of Instruction for Dairy Inspectors*A. C. ANDERSON, *Chairman*

I. C. WELD

H. E. VAN NORMAN

J. A. GAMBLE

C. E. REED

ERNEST KELLY

*Feeding Standards for Milk Production*C. LARSON, *Chairman*

A. A. BORLAND

C. H. ECKLES

E. S. SAVAGE

HELMER RABILD

C. C. HAYDEN

A. C. RAGSDALE

H. P. DAVIS

*Score Cards for Dairy Products*J. H. FRANDSEN, *Chairman*

ERNEST KELLY

H. B. ELLENBERGER

W. P. B. LOCKWOOD

S. C. THOMPSON

W. W. FISK

B. W. HAMMER

J. L. SAMMIS

L. A. ROGERS

O. F. HUNZIKER

J. A. GAMBLE

*Methods for Conducting Student Dairy Products Judging Contest*WILLIAM WHITE, *Chairman*

E. S. GUTHRIE

W. P. B. LOCKWOOD

J. H. FRANDSEN

A. W. RUDNICK

H. F. JUDKINS

S. C. THOMPSON

*State and National Brands for Butter and Cheese*W. W. FISK, *Chairman*

C. LARSEN

C. E. LEE

N. W. HEPBURN

A. W. RUDNICK

N. D. CHAPPELL

*Cream Grading*J. H. FRANDSEN, *Chairman*

G. S. HINE

F. W. BOUSKA

C. W. LARSEN

GEORGE B. CAINE

J. A. CLUTTER

G. L. MARTIN

## COMMITTEE APPOINTMENTS

*Legal Standards for Ice Cream*

H. A. RUEHE, <i>Chairman</i>	E. F. GOSS
R. M. WASHBURN	W. W. FISK
O. F. HUNZIKER	

*Official Methods for Testing Butter for Butter Fat*

H. C. TROY, <i>Chairman</i>	C. E. GRAY
R. H. SHAW	

*Cost of Production*

OSCAR ERF, <i>Chairman</i>	G. F. WARREN
F. A. PEARSON	A. C. ANDERSON
ERNEST KELLY	

*Constitution and By-Laws*

C. H. ECKLES, <i>Chairman</i>	W. J. FRASER
FRED RASMUSSEN	R. A. PEARSON
O. F. HUNZIKER	J. H. FRANDSEN
M. MORTENSEN	

## DAIRY NOTES

J. W. HENDRICKSON

*University of Nebraska*

The Dairy Division at Washington, D. C., gives a report of the following changes:

*Mr. C. J. Babcock*, assistant market milk specialist, has been reappointed in the Dairy Division and has been assigned to dairy sanitation investigations in the market milk section. Mr. Babcock is a graduate of Ohio State University.

*Dr. N. R. Blatherwick*, who has been engaged on investigations in the physiology of milk secretion since 1915, conducted by this Division at its dairy farm at Beltsville, Maryland, has resigned to take charge of a research laboratory at Santa Barbara, California, which is being conducted in connection with a hospital where studies are being made of nutrition diseases, under the auspices of the Rockefeller Institute.

*Mr. P. A. Clemmer*, who has been engaged in bacteriological investigations of milk since 1915, has resigned to go into business in Pennsylvania.

*Mr. L. B. Cook*, market milk specialist since 1912, who has recently conducted milk sanitation investigations in the vicinity of Grove City, Pennsylvania, has resigned to accept the position of manager of a milk plant in Kane, Pennsylvania.

*Mr. A. O. Dahlberg*, dairy manufacturing specialist since 1912, engaged in research problems in dairy manufacture, has resigned to accept a position with the California Central Creameries.

*Mr. C. S. Leets*, assistant market milk specialist since 1917, has resigned to accept a position as field agent and inspector for the New Orleans Pure Milk Company, New Orleans, Louisiana.

*Mr. R. J. Posson*, market milk specialist, has been transferred from the Washington office to the Dairy Division office at Salt Lake City, to take the place of C. F. Hoyt, resigned. Mr. Posson will carry on the market milk work in the western states, which deals principally with the California State Department of Agriculture at Sacramento.

*Mr. Geo. B. Taylor*, market milk specialist of the Dairy Division since 1915, has resigned to accept the position of chemist and bacteriologist with Geo. M. Oyster's "Chestnut Farms Dairy," Washington, D. C.

*Mr. P. A. Campbell*, has been appointed for dairy extension work in the State of Connecticut, conducted coöperatively by the Dairy Division, United States Department of Agriculture, and the extension department, Storrs, Agricultural College, Storrs, Connecticut. Mr Campbell received his B. S. degree from New Hampshire State College, and his Master's degree from Iowa State College. He served as professor of animal husbandry, University of Maine, 1905-1913; as manager of Hillside Dairy Company, Dixville Notch, New Hampshire; and recently was manager of Ayredale Stock Farm, Bangor, Maine.

We are pleased to report that Prof. C. L. Roadhouse of the department of dairy industry, University of California, has under process of erection a fine new dairy building which will when completed add much to the efficiency of both the teaching and the experimental work to be carried on at that institution.

Professor Roadhouse also reports some changes that have taken place in his staff of co-workers.

*Mr. S. L. Denning*, instructor in the dairy industry division at the University of California, resigned in January to take a position with the Premier Machinery Company, San Francisco, California.

*Mr. H. S. Baird*, assistant professor of dairy industry, University of California, has a leave of absence, and has taken a position as superintendent of manufacture with the Northern California Milk Producers Association at Sacramento, California.

*Mr. G. D. Turnbow*, formerly dairy extension specialist at University of Wyoming, has accepted a position as assistant professor of dairy industry at University of California. Mr. Turnbow took up his new duties on February 21, 1920.

*Jean Prescott Adams*, director of food economics for Armour and Company, gives the following points relative to the care, preparation and use of cheese:

#### THE PRINCIPLES IN CHEESE COOKERY

With cheese in such prominence in the dietary of the individual, the housewife will be anxious to acquaint herself with some of the outstanding points in relation to cookery and digestion of this most important product.

When cheese is first purchased, a few minutes should be taken to properly care for it. Wrap it either in oiled paper or in a wet tea towel and store it so as to avoid any possible contamination from strong flavors or odors.

Being a ready-to-eat product, cheese in cookery is heated merely to melt it and incorporate it with the rest of the mixture. This operation does not require

high heat. And, too, being high in protein content, cheese must be cooked at a low temperature in order to attain a resulting product that will be easily digested. Therefore, in most made cheese dishes, the double boiler is employed. By this method of radiation, a cheese dish may be prepared without direct contact of high heat. If necessity demands a direct flame, the flame should be low and the time of cooking should be short.

American cheese serves many purposes. The effect of creamed cheese may be obtained by adding grated cheese to small amount of cream. Cheese may be used in desserts. Its flavor is well liked in pastries and in salads it is unsurpassed. Caution must be exercised in the adding of cheese to acid material. It is advisable in made dishes where cheese and milk are to be mixed with acid, as acetic in vinegar, to dilute the vinegar with water, and then mix it with the flour which is generally used as a thickening agent in cheese dishes.

Cheese left over may be used in extending cheese flavor in vegetable dishes, mashed potatoes or in pastries. All unused cheese, if of sufficient amount, may be coated with paraffin and stored away for future use.

*Dr. E. S. Guthrie*, of Cornell University, dairy department, furnishes the following news items:

*Prof. H. C. Troy*, who has been on sabbatic leave during the first semester of the 1919-1920 college year, has returned to his duties at Cornell. Professor Troy was collaborating in some work with Mr. Timothy Mojonnier, of Mojonnier Brothers Company, Chicago, Illinois.

*Prof. F. W. Bouska* of the American Creamery Butter Manufacturers' Association, visited Cornell in February, and addressed the dairy students.

*Prof. Fred Rasmussen*, Secretary of Agriculture of Pennsylvania, was a Farmers' Week speaker at Cornell.

The new additions to the staff at Cornell this year are: Mr. M. P. Moon, H. B. Neville, W. V. Price, L. E. Smith, and M. B. Robinson. These men were all former students and are now taking graduate work. Mr. Moon and Mr. Neville were in the sanitary corps in the army. Mr. E. Pittman, of the University of Kentucky, is also a new addition to the department of dairy industry, at Cornell, and is taking graduate work.

*Prof. James D. Brew*, a graduate of Cornell, later of the experiment station staff of Geneva and Illinois, and later in the employ of the Nestle's Food Company, is now assistant professor of extension teaching in the dairy department, at Cornell.

*Mr. G. Clayton Dutton*, for several years an extension instructor of the department of dairy industry, at Cornell, specializing in cheese, is now in commercial work in Vermont.



*Mr. C. R. Owens* and *Mr. L. D. Spink*, of the department of farms and markets of New York State, assisted in the instruction of the winter course in dairying.

The following extract is taken from a report of the Glass Container Association of America:

There are obvious reasons why the public want glass:

1. The fact that people can see the cream line and the contents protects them against fraud by those milk dealers who might be disposed to put a very thin or even skimmed milk into the paper container without detection.
2. The same reason enables the public to know that their milk is clean.
3. The glass bottle adapts itself readily to machinery.
4. Bacteria growth is less in glass.
5. There is no foreign taste or flavor to milk in glass bottles.
6. Milk can be iced, handled and kept more easily in glass.
7. Glass is by far the cheapest of all containers.
8. The glass bottle is the most sterile of all containers.

#### MINERAL MATTER AND MILK

Experiments conducted by the Bureau of Animal Industry, United States Department of Agriculture, with dairy cows are showing an important relation between milk secretion and certain mineral substances. Feeding compounds of phosphorus and calcium have resulted in a decidedly beneficial effect on the milk flow in both quantity and fat content.

It has been shown also that a deficiency of phosphorus in dairy rations has a detrimental effect on milk secretion of cows and growth of calves. A remedy was found in the addition of sodium phosphate to rations deficient in phosphorus. The work is being continued.

#### USE FOR WASTED WHEY

Development of a method of utilizing whey as a human food is the object of work now in progress in the Bureau of Animal Industry, United States Department of Agriculture. It is thought probable that whey represents a greater actual loss of food than skim milk—which now has become an important by-product in the dairy industry—because its feeding value is not generally recognized. Cheeses have been made from whey, but the demand for them has been limited. The use of these cheeses might be extended if their value for cooking could

be brought to the attention of housekeepers. Investigations of the use of whey solids as poultry feed also have been begun.

Considerable work has been done on the development of casein for use in waterproof glue, and a casein of low ash and acid has already been produced.

A method of producing casein from buttermilk is also being worked out. By use of a solvent to extract the fat from the buttermilk, small lots of casein have been made, and this product was found to be of general good quality and low in fat and ash, but it had the objection of dissolving slowly.

#### HOW FOREIGN COMPETITION AFFECTS DAIRY INDUSTRY

To meet foreign competition, dairy farmers of the United States must be able to produce a better quality of product and produce and market it more economically and more efficiently, according to specialists in the Bureau of Markets, United States Department of Agriculture.

Arrivals of shipments of Danish butter are already affecting prices on the New York city market. Argentina is producing nearly three times the amount of butter and cheese consumed, and some of the surplus may be expected to come to this country or compete with our products in foreign countries. Before the war Siberia was rapidly extending its dairy industry, and when conditions become settled in that country it may be expected to come back as a factor in the world's market. Recently there have been signs of interest in dairying in South Africa, and the industry as developed in New Zealand and Australia must be reckoned with.

If the dairy products manufactured in the United States are of a better quality than those from other countries they need not fear competition. Canada's cheese industry illustrated this. A strict system of government supervision in the training of cheese makers, in the operating of the factories, and in the grading, marketing, and exporting of the product exists there. This has tended toward an improvement in the quality of Canadian cheese until it ranks with the finest on the English markets.

The dairy industry in Argentina has grown rapidly since the beginning of the war. Before the war butter exports from that country totaled 3262 tons a year; in 1918 they were five times that. Cheese

exports were far exceeded by the imports in 1913. Now the conditions are reversed—over 6000 tons of cheese being exported in 1918.

Today most of these exports are going to European markets, but should conditions become favorable it may be expected that some of them will come to this country. The Bureau of Markets warns dairy-men to be prepared to meet this competition.

# CLARIFICATION OF MILK<sup>1</sup>

CHARLES E. MARSHALL AND E. G. HOOD

TOGETHER WITH ARTHUR N. JULIAN, S. G. MUTKEKAR AND MAX S. MARSHALL

*Massachusetts Agricultural Experiment Station, Amherst, Massachusetts*

That the clarifier is capable of performing certain functions in cleaning milk and modifying its fermentations is abundantly apparent to any close student of the process. Whether it has reached the acme of its powers is still to be demonstrated. In the present article it is the purpose of the writers to convey some of the causes for its influence upon microbial activities. It is not felt, however, that all of these have been determined.

In Bulletin 187, Massachusetts Agricultural Experiment Station, and in an article presented before the American Public Health Association<sup>2</sup> at New Orleans, a summation of what the clarifier does is obtainable and it need be repeated here only in a form to serve the purpose of presenting an analysis and proof. In order to classify the results without furnishing unnecessary and too many accumulated data, only illustrative experimental evidence will be offered throughout this article.

## I. VARIATIONS IN THE MICROBIAL CONTENT OF MILK AFFECTING CLARIFICATION

It is a well established fact that milks differ very widely in their microbial content, consequently the reaction of milk to the clarifier must be variable. This has been set forth in one way and another throughout Bulletin 187 and especially is it set forth by the fact that the numbers of microorganisms eliminated are subject to great variations which are due to many factors. The fermentations incident thereto likewise are not uniform and are widely variable.

<sup>1</sup> This article will be published later by the Massachusetts Agricultural Experiment Station as Part II of Bulletin 187.

<sup>2</sup> Studies in the clarification of milk, by C. E. Marshall, E. G. Hood, S. G. Mutkekar, John Yesair and Max Marshall.

## II. AN ANALYSIS OF SOME DIFFERENCES EXISTING BETWEEN UNCLARIFIED AND CLARIFIED MILK

1. *Naked eye appearances.* To the worker the differences between unclarified and clarified milk become more and more evident as he studies innumerable samples by contrast or comparison. Sometimes they are slight, sometimes very noticeable to the naked eye. They find their explanation in the evidence which follows.

2. *Microbial evidences.* It became necessary to utilize distinct species of microorganisms for satisfactory results because platings and isolations from unclarified and clarified samples of milk proved unsatisfactory in our hands in reaching an analytical determination of microbial values.

TABLE 1

*Illustrative test; per cent of organisms eliminated by the clarifier*

<i>Oidium lactis</i> .....	99+
<i>Saccharomyces cerevisiae</i> .....	99+
<i>B. tumescens</i> .....	66+
<i>B. subtilis</i> .....	47+
<i>B. coli</i> .....	35+
<i>Strept. pyogenes</i> .....	20+
<i>Strept. lacticus</i> .....	24+

An arrangement from the largest to the smallest forms of organisms will convey the significance of the action of the centrifugal force of the clarifier.

While these numbers may vary considerably, it is true that colonies or organisms in masses and large organisms are more readily cast out than small organisms, due probably to the relation of the surface area of the cell to the cell volume. This corresponds exactly with the work of any centrifuge.

3. *Proteolysis.*<sup>3</sup> There is every indication, too, that proteolysis takes place to a greater extent in unclarified than in clarified milk. This is attributable to the lactic direction given to fermentation by the clarifier. Particular attention will be given to this "directive influence" under "Causes for Differences" which follows

<sup>3</sup> These determinations were made by Mr. S. G. Mutkekar.

later. Out of the analyses of ten samples, the writers will offer only one inasmuch as all are very much the same. The Kjeldahl method was employed in the determinations.

4. *Acidity.* A difference in acidity has been recognized by several workers whose publications have been noted in Bulletin 187. There is a slight increase in the clarified samples. This increase is uniform and harmonizes with carbon dioxide findings which will be given later and with the lactic directive influence already suggested.

TABLE 2  
*Illustrative sample*

AGE OF MILK	TOTAL N IN 100 CC. SERUM		PROTEIN PER 100 CC. SERUM		INCREASE OF SOLUBLE PROTEIN	
	A	B	A	B	A	B
<i>days</i>						
6	0.1317	0.1359	0.823	0.849		0.026
7	0.1345	0.1352	0.841	0.844		0.003
8	0.1303	0.1387	0.814	0.867		0.053
9	0.1310	0.1429	0.819	0.893		0.074
10	0.1275	0.1331	0.797	0.832		0.035
11	0.1303	0.1450	0.814	0.906		0.092
12	0.1331	0.1401	0.832	0.876		0.044
13	0.1457	0.1401	0.911	0.876	0.035	
14	0.1436	0.1471	0.897	0.919		0.012
15	0.1485	0.1583	0.928	0.989		0.061

A, clarified milk; B, unclarified milk.

Only one illustrative table will be inserted as representative of thirteen similar determinations made. The method of determination is included that the reader may give proper interpretation to the results.

*Determination of acidity in milk serum.* 100 cc. of milk were put in flasks which were held at temperature ranging from 12°–14°C. until the milk had curdled—usually three to five days. The milk was then filtered and serum obtained. An aliquot of 10 cc. of this serum was then pipetted into a flask and diluted to 100 cc. with distilled water. Two or three drops of phenolphthalein were then added and the titration accomplished by decinormal NaOH.

5. *Gas production.* The determination of the total amount of gas to be found in clarified and unclarified milk has not yet been attempted. However, the amount of carbon dioxide formed has been accurately measured by Prof. Arthur N. Julian who reports the results as shown in tables 4 and 5.

*Methods used.* In general, the method used in the following determinations of  $\text{CO}_2$  production in clarified and unclarified milk is that employed by E. Truag of the Soils Department, Wisconsin Experiment

TABLE 3  
*Illustrative sample*

DATE	AGE OF MILK	N/10 $\text{NaOH}$ USED	
		B	A
1918	days	cc.	cc.
January 28.....	4	6.00	5.94
January 29.....	5	5.80	5.80
January 30.....	6	5.82	5.88
January 31.....	7	6.14	6.50
February 1.....	8	6.76	6.48
February 2.....	9	6.74	6.82
February 3.....	10	6.70	6.80
February 4.....	11	6.90	7.00
February 5.....	12	6.82	7.00
February 6.....	13	7.10	7.52
February 7.....	14	7.40	7.52
February 10.....	17	7.40	7.84
February 11.....	18	7.54	8.10
February 12.....	19	7.90	8.30

A, clarified milk; B, unclarified milk.

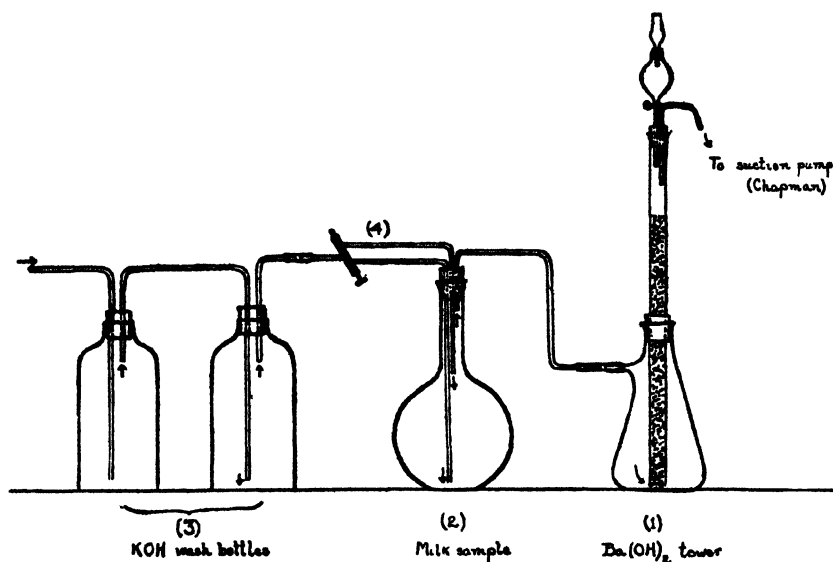
Station, in his work on  $\text{CO}_2$  in soils. (J. Ind. and Eng. Chem., vii, no. 12, December, 1915).

Special adaptations to the problem in hand may be mentioned as follows, the accompanying diagram illustrating the arrangement of apparatus.

This apparatus was set up in duplicate, so that the clarified and unclarified samples of milk were receiving treatments as nearly as possible identical.

Two wash bottles containing strong  $\text{KOH}$  (3) and broken glass were employed to deliver  $\text{CO}_2$ -free air to the flask containing the milk

sample (2), then a two-way stopcock (4), one tube of which extended only to the surface of the milk in flask (2), while the second arm delivered air to the bottom of the flask. The first of these tubes (delivering air to the surface) was used in making the first two determinations of each experiment, representing the free evolution of gas; the arm extending to the bottom was employed in displacing the  $\text{CO}_2$  gas held in solution, in making the third determination, approximating "total gas."



A third pair of wash bottles, identical with that in diagram, was employed in the preliminary filling of the Truag tower and suction flask (1) with  $\text{CO}_2$ -free air.

No attempt was made to determine how closely the "approximate total gas" of the third determination approached actual total gas, one and one-half hours' suction being chosen arbitrarily as a reasonable standard time for both samples, hence valuable only for comparative purposes.

The usual precautions were observed in the matter of uniformity of milk, safeguards against contamination from  $\text{CO}_2$  of the air at all steps, etc.



The following table presents the results of ten experiments, five on raw commercial and five on raw certified milk, figures representing CO<sub>2</sub> in terms of grams per 1200 cc. milk employed.

TABLE 4  
*Market milk*

DETERMINATION	SAMPLE I		SAMPLE II		SAMPLE III		SAMPLE IV		SAMPLE V	
	Clarified	Unclarified	Clarified	Unclarified	Clarified	Unclarified	Clarified	Unclarified	Clarified	Unclarified
1. 18- 24 hrs.	24 hrs. 0.0108	24 hrs. 0.0035	24 hrs. 0.0103	24 hrs. 0.0050	24 hrs. 0.0024	24 hrs. 0.0024	24 hrs. 0.0132	24 hrs. 0.0086	27 hrs. 0.0130	27 hrs. 0.0092
2. 42- 48 hrs.	48 hrs. 0.0194	48 hrs. 0.0176	44 hrs. 0.0117	44 hrs. 0.0035	48 hrs. 0.0139	48 hrs. 0.0123			43 hrs. 0.0125	43 hrs. 0.0051
3. 50- 60 hrs.	60 hrs. 0.0755	60 hrs. 0.0563	53 hrs. 0.0290	53 hrs. 0.0372	60 hrs. 0.0161	60 hrs. 0.0147	40 hrs. 0.0227*	40 hrs. 0.0205*	53 hrs. 0.0257	53 hrs. 0.0255
Totals	0.1057	0.0774	0.0510	0.0457	0.0324	0.0294	0.0359	0.0291	0.0512	0.0398

\* Total at 40 hours to save sample from going over top; excessive gas formation.

TABLE 5  
*Certified milk*

DETERMINATION	SAMPLE I		SAMPLE II		SAMPLE III		SAMPLE IV		SAMPLE V	
	Clarified	Unclarified	Clarified	Unclarified	Clarified	Unclarified	Clarified	Unclarified	Clarified	Unclarified
1. 18- 24 hrs.	12 hrs. 0.0015	12 hrs. 0.0011	24 hrs. 0.0059	24 hrs. 0.0018	24 hrs. 0.0062	24 hrs. 0.0020	18 hrs. 0.0059	18 hrs. 0.0015	24 hrs. 0.0066	24 hrs. 0.0020
2. 42- 48 hrs.	36 hrs. 0.0090	36 hrs. 0.0090	48 hrs. 0.0152	48 hrs. 0.0059	48 hrs. 0.0266	48 hrs. 0.0185	42 hrs. 0.0145	42 hrs. 0.0057	42 hrs. 0.0134	42 hrs. 0.0046
3. 50- 60 hrs.	60 hrs. 0.0162	60 hrs. 0.0279	60 hrs. 0.0169	60 hrs. 0.0172	60 hrs. 0.0460	60 hrs. 0.0583	50 hrs. 0.0480	50 hrs. 0.0526	50 hrs. 0.1415	50 hrs. 0.1078
Totals	0.0267	0.0380	0.0380	0.0249	0.0788	0.0788	0.0684	0.0598	0.1615	0.1144

From the results of Professor Julian the writers are led to interpret the increased amount of carbon dioxide in clarified milk as due to the stimulation given to lactic fermentation which has already been referred to and the reasons for which will appear in connection with other considerations.

6. *Methylene blue reduction.* The reduction of methylene blue is so little understood that to draw any particular conclusions is precarious. Some have claimed that clarified milk has reduced methylene blue more quickly than unclarified milk and others that unclarified acts more rapidly. The illustrative tests inserted will indicate that the unclarified milk has reduced methylene blue more rapidly than the clarified although constancy does not exist throughout. The limited number of tests presented as illustrative furnish a fair notion of the general results secured.

*Method employed for reduction of methylene blue.* Methylene blue: 5 cc. concentrated alcoholic solution and 195 cc. distilled water. 2 cc. of this solution is added to 50 cc. of milk and the mixture well shaken until the color is uniform. Better to make this mixture in sterile flasks and then pour into the test bottles. These bottles are then kept in the incubator at 37°C. and the changes noted from time to time.

7. *Character of curd.*—It has been convincing to ascertain in a large percentage of cases that the casein obtained by filtration from unclarified and clarified milk followed a distinctive course in decomposition. The casein deposit from the unclarified milk produced molds abundantly and seemed to undergo proteolysis readily and rapidly; that from the clarified milk seemed much freer from molds and proteolysis and followed the course as witnessed in cheese curd when acid has been developed. This bears testimony to the starter-effect of the clarified which has been reverted to in this discussion quite frequently.

In securing the above curds, the milk samples before and after clarifying were set aside in a suitable place. In our work a room having a constant temperature of about 17°C. has been used. After the milk samples had curded, they were carefully filtered through sterile filter paper. The precipitated curd, covered over, was held on the paper in the funnel and the changes watched.

This procedure proved to be one of the most striking crude methods of noting the differences between unclarified and clarified milk. If care is exercised, it is a method which may be easily utilized without laboratory facilities.

TABLE 6

*Illustrative tests; reduction of methylene blue; certified milk*

NUMBER OF TEST	DATE	SAMPLE	AGE OF MILK	TEMPER- ATURE OF HOLDING	TIME OF STARTING REDUCTION		TIME OF COMPLETE REDUCTION		TOTAL TIME OF REDUCTION		BACTERIA PER CUBIC CENTI- METER
					Hours	Minutes	Hours	Minutes	Hours	Minutes	
I	4/15	A	Once	deg. C. 20-22	6	37	3	5	9	42	3,600
		B	Once	20-22	6	0	3	0	9	0	5,500
	4/16	A	24	20-22	0	49	0	34	1	23	8,000,000
		B	24	20-22	1	23	0	22	1	45	7,200,000
	4/17	A	48	20-22	1	15	0	30	1	35	60,000,000
		B	48	20-22	1	0	0	8	1	8	84,000,000
II	4/20	A	Once	20-22	6	12	2	50	9	2	4,800
		B	Once	20-22	5	45	2	30	8	15	5,900
	4/21	A	24	20-22	1	58	0	19	2	17	7,300,000
		B	24	20-22	1	18	0	23	1	41	9,800,000
	4/22	A	48	20-22	0	17	0	11	0	28	
		B	48	20-22	0	21	0	10	0	31	
III	4/23	A	Once	20-22	4	45	0	54	5	39	250,000
		B	Once	20-22	4	30	1	0	5	30	400,000
	4/24	A	24	20-22	0	22	0	3	0	25	63,000,000
		B	24	20-22	0	15	0	5	0	20	121,000,000
	4/25	A	48	20-22	0	32	0	10	0	42	189,000,000
		B	48	20-22	0	28	0	10	0	38	145,000,000
IV	5/2	A	Once	20-22	7	15	0	30	7	45	4,000
		B	Once	20-22	6	10	1	15	7	25	8,000
	5/3	A	24	20-22	0	58	0	12	1	10	20,000,000
		B	24	20-22	0	47	0	8	0	55	12,000,000
	5/4	A	48	20-22	0	57	0	19	1	16	49,000,000
		B	48	20-22	0	33	0	26	0	56	57,000,000
V	5/10	A	Once	20-22	11	15	4	15	15	30	1,600
		B	Once	20-22	10	45	4	0	14	45	4,100
	5/11	A	24	20-22	4	15	3	22	7	37	320,000
		B	24	20-22	4	0	3	0	7	0	450,000
	5/12	A	48	20-22	1	40	0	15	1	55	21,000,000
		B	48	20-22	0	25	0	3	0	28	46,000,000
VI	5/16	A	Once	20-22	4	40	1	25	6	5	1,020,000
		B	Once	20-22	4	15	1	50	5	55	1,520,000
	5/17	A	24	20-22	0	7	0	17	0	34	219,000,000
		B	24	20-22	0	4	0	3	0	7	320,000,000
	5/18	A	48	20-22	*						
		B	48	20-22							

A, clarified milk; B, unclarified milk.

\* Milk coagulated.

The laboratory tests indicate that clarified and unclarified milk may be differentiated. The surface of the unclarified sample should reveal an abundant growth of molds while the clarified sample will appear quite free from molds. If this is true of molds, it is easily understood how yeasts and large microorganisms have been eliminated in clarifying. The influence exerted by these organisms upon milk may be measured in a degree by the study of association.

These tests with the curds and with the samples of unclarified and clarified milks are visible, striking and convincing, although furnishing only a crude interpretation.

### III. THE LEADING CAUSES OF SOME OF THE DIFFERENCES BETWEEN UNCLARIFIED AND CLARIFIED MILK

1. *Selective action.* By selective action is meant the power of the clarifier to pick out the heavier organisms in greater percentage than the lighter organisms. This power has been reverted to in Bulletin 187 and again at the beginning of this paper in which the rate of elimination of various organisms by the clarifier is given. In this respect the clarifier corresponds closely to a centrifuge.

Using an International Instrument Company's No. 1-B centrifuge with a speed of 2250 revolutions per minute and suspending the microorganisms in milk the following results were secured.

Comparing the work of the centrifuge with that of the clarifier above, it would be approximately true to say that the centrifuge running ten to fifteen minutes gives about the same results as the clarifier gives running at 8000 revolutions per minute and in which the passage of milk is effected in about seven seconds.

The clarifier has demonstrated its efficiency in casting out dirt particles which have a specific gravity greater than milk. The larger microorganisms apparently respond to its action very readily as is shown in table 1. This is due to the specific gravity and likewise to the relation of surface to cell-volume. The smaller the organism, the greater the extent of surface is

to its cell-volume although the density of the cell-contents may be the same as in the larger organism or may be different. It is also a matter of common observation among microbiologists that microorganisms are not constant in their density.

TABLE 7  
*Illustrative tests*

TIME	MICROORGANISMS		
	<i>B. subtilis</i> per loop	<i>Strept. lacticus</i> per loop	<i>Oidium lactis</i> spores per loop
<i>minutes</i>			
At start	3561	164	180
5	1017	121	3
10	349	104	1
15	84	84	1
20	48	73	
25	13	49	
30	8	45	
Per cent removed....	99.8	72	100, at end of 20 minutes

TABLE 8

	PER CENT OF TOTAL ELIMINATED IN		
	First Clarification	Second Clarification	Third Clarification
<i>Sacch. cerevisiae</i> .....	99.2	99.6	99.9
<i>Strept. lacticus</i> .....	20.0	55.1	85.1

Comparing two organisms, *Saccharomyces cerevisiae*, a large organism, and *Streptococcus lacticus*, a small organism, in whole milk after passage through the clarifier three times will give some idea of the relative results. The removal of such organisms as *Oidium lactis*, yeasts and large bacteria has a decided tendency to disturb the germ-equilibrium which, in the case of milk, results in the lactic organisms, which are very small and do not colonize readily, dominating the field and thus giving rise to quickened lactic fermentation. When operating together with two other factors, associative influences and distribution, which

will now be considered, it is readily seen that a combination of forces is operative in a clarifier, each one of which has some influence, although difficult to determine quantitatively.

2. *Associative influences.* It will suffice, in this connection, to confine ourselves to a single study which will set forth our ideas, although many phases of this particular subject has been investigated.

*Preparation.* Forty 300-cc. flasks containing 100 cc. of litmus skimmed milk each were made ready by the usual methods. The milk was sweet (no acid could be detected) skimmed market milk and of the same lot. These flasks were arranged in duplicate series. Two flasks were held as sterile checks and two used for *Strept. lacticus* control. Three series of six flasks each in duplicate were treated as indicated in table 9. The inocula consisted of a culture of *Strept. lacticus* diluted so that each drop from a Roux pipette contained 200 organisms; of *B. subtilis* diluted in the same manner but each drop contained only 25 organisms; of *Oidium lactis* spores diluted in the same manner but each drop contained 500 spores. The flasks were arranged and inoculated according to the following scheme with the results indicated opposite each series.

Under "selection" it is evident that there is a tendency to disturb the numerical relationships or equilibrium of micro-organisms present in milk or any other fluid serving as a menstruum for fermentation. This disturbed relationship, therefore, must result in an altered or modified fermentation. For instance, it is easily demonstrated that the presence of *B. subtilis* in association with *Strept. lacticus* hastens the action of the latter. It is also easily demonstrated and well known that with the rapid development of *Strept. lacticus* in the presence of *Oidium lactis*, the growth of *Oidium lactis* is hastened. Further, it can be easily demonstrated that the growth of *B. subtilis* and *Strept. lacticus* stimulates the *Oidium lactis* beyond that of the *Strept. lacticus* alone. Now if a centrifuge or clarifier is introduced in a mixed culture of *Strept. lacticus*, *B. subtilis* and *Oidium lactis*, in milk, the *Oidium lactis* is thrown out to such a degree that when *Strept. lacticus* develops only a colony here and there will be found; *Oidium lactis* growth becomes practically negligible.

TABLE 9  
*Illustrative associative study*

	FLASK NUM- BER	INOCULA		RESULTS
Check Series I	1	Sterile—none		Remained sterile
	2	Sterile—none		
<i>Strept. lacti- cus</i> , Series II		<i>Strept. lacticus</i>		Curded in 72 hours with litmus re- duction normal
	1	1 drop		
	2	1 drop		
<i>Strept. lacti- cus</i> + <i>B. subtilis</i> , Series III		<i>Strept. lacticus</i>	<i>B. subtilis</i>	Whole series fin- ished curding in 53 hours. Litmus changes slightly altered. Series flask No. 6 curded in 51 hours or 2 hours before se- ries flask No. 1. Curding proceed- ed from 6 to 1
	1	1 drop	+ 1 drop	
	2	1 drop	+ 4 drops	
	3	1 drop	+ 8 drops	
	4	1 drop	+ 12 drops	
	5	1 drop	+ 16 drops	
	6	1 drop	+ 20 drops	
<i>Strept. lacti- cus</i> + <i>Oi- dium lactis</i> , Series IV		<i>Strept. lacticus</i>	<i>Oidium lactis</i>	Whole series curded together in 72 hours same as Series II. <i>O. lac- tis</i> does not affect cultures; does not grow much be- fore acid forms. Growth of <i>O. lac- tis</i> normal and vigorous. De- struction of acid by <i>O. lactis</i> was normal. Litmus changes followed lactic fermenta- tion as in Series II till <i>O. lactis</i> reduced it almost permanently.
	1	1 drop	+ 1 drop	
	2	1 drop	+ 4 drops	
	4	1 drop	+ 8 drops	
	4	1 drop	+ 12 drops	
	5	1 drop	+ 16 drops	
	6	1 drop	+ 20 drops	

TABLE 9—Continued

	FLASK NUM- BER	INOCULA			RESULTS
		<i>Strept. lacticus</i>	<i>B. subtilis</i>	<i>Oidium lactis</i>	
<i>Strept. lacti- cus</i> + <i>B. subtilis</i> + <i>Oidium lac- tis</i> , Series V	1	1 drop +	1 drop +	1 drop	Whole series curded in 56 hours or 3 hours later than Series III. Series flask No. 6 curded in 53 hours or 3 hours earlier than series flask No. 1. Curding proceed- ed from 6 to 1. Growth of <i>O. lac- tis</i> more rapid and more vigor- ous than in Series IV. Destruction of acid by <i>O. lac- tis</i> also more rap- id than in Ser- ies IV. Litmus changes same as in Series IV but much more pro- nounced.
	2	1 drop +	4 drops +	4 drops	
	3	1 drop +	8 drops +	8 drops	
	4	1 drop +	12 drops +	12 drops	
	5	1 drop +	16 drops +	16 drops	
	6	1 drop +	20 drops +	20 drops	

One drop *Streptococcus lacticus* = 200 organisms; 1 drop *Bacillus subtilis* = 25 organisms; 1 drop *Oidium lactis* = 500 organisms.

Room temperature 17° to 25°C. maintained.

Further, should *B. subtilis* be removed to such an extent that its stimulating influence upon *Strept. lacticus* and *Oidium lactis* is submerged, then it can readily be seen that the fermentation produced by *Strept. lacticus* and *Oidium lactis* is retarded. In such a combination or mixed culture in milk as just named, *Strept. lacticus* is eliminated by the clarifier in a much less degree, 24 per cent, than is either of the other organisms, *Oidium lactis*, 99 + per cent, of *B. subtilis*, 47 + per cent. The result will be that *Strept. lacticus* left behind is likely to act very much as a starter or become actually conspicuous by its retention and fermentation. The other two organisms, *B. subtilis* and *Oidium lactis*, lose a large share of their significance.



Such combinations of microorganisms or mixed cultures can easily be followed to a point where the individualities of the organisms sink beyond recognition; in other words, associative influences may be followed as long as the species present manifest to the observer their individual action; then the analysis, or synthesis as the case may be, loses its clear definition and the observer becomes confused, no individuality of species can be determined. This evidence, however, has its bearing in connection with some of the results obtained from the use of the clarifier with certified or market milk,—a modification of the fermentation is evident in the majority of samples but this modification is extremely difficult to interpret. Differences in fermentation are detected many times between the unclarified and clarified milk, yet so complex and so variable are the conditions and the germ-content that an understanding must be through indirect channels and influences. So difficult, in fact, is it to determine these elemental differences, or factors involved, in terms of definite, positive and known data that it seems necessary at present to resort to this inferential study of selection and association if a clear idea is to be gained of what actually transpires in the use of the clarifier.

3. *Distributive action.* It has been possible to demonstrate the distributive action of the clarifier in several ways.

a. The increase in the number of microorganisms in market milk after clarification as revealed in Bulletin 187 is of common experience. In fresh or certified milk where colonization is not permitted to take place so freely the tendency is toward reduction in numbers.

b. When *B. coli* is placed in glucose agar and the agar passed through the clarifier, the colonies resulting from the increased number of foci and the formation of gas bubbles at these points give a graphic illustration of what has taken place in the clarifier—a colony apparently is broken into innumerable fragments.

c. If a little oil is placed in water and the mixture passed through the clarifier, the mixture becomes a very fine emulsion.

All of these experiences confirm the influence of the clarifier in causing the breaking up of colonies or groups of microorgan-

isms in milk and causing their distribution throughout the mass.

4. *Aeration.* Air is taken into the clarifier bowl and mixed with the milk. It also finds its way in when impinging against the surface of the receiving pan as it emerges from the bowl. This is not a constant amount but the ratio as determined is about 7 to 3.

If milk is thoroughly aerated while clarifying, it is easily understood how the growth of microorganisms is affected. The action in clarification, so far as can be detected, favors lactic fermentation.

5. *Unknown influences.* The writers feel that they have not yet encompassed all of the agencies at work in the clarifier. This attitude results from certain manifestations which the writers have not yet been able to attack effectively or are so concealed that they frustrate attempts.

#### SUMMARY

1. The clarifier by its action modifies the fermentations of milk by its influence upon the germ-content.

2. These differences in the fermentation of clarified and unclarified milk are made manifest by

(1) Alterations visible to the naked eye. These are not transferable to paper but are due to the factors in part indicated below.

(2) Microbial evidences such as the growth of *Oidium lactis* upon the surface of the milk or the lactic fermentation.

(3) Proteolysis.

(4) Acidity.

(5) Gas production.

(6) Methylene blue reduction.

(7) Character of curd.

3. The causes for these differences cited under 2 are

(1) Selective action of the clarifier.

(2) Associative influences resulting from disturbed germ-content.

(3) Distributive action of clarifier.

(4) Aeration effected by clarifier.

(5) Causes at present unknown or undeterminable.

# THE RELATION OF AGE OF DAM TO OBSERVED FECUNDITY IN DOMESTICATED ANIMALS

## I. MULTIPLE BIRTHS IN CATTLE AND SHEEP<sup>1</sup>

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### INTRODUCTION

The fact that the present paper is concerned with fecundity as affected by the age of the dam and that the term fecundity is not always employed as indicating the same function, necessitates a definition of the term. This necessity is further emphasized by past usage in the literature on the physiology of reproduction and in stock journals, where the words fecundity and fertility are often used interchangeably.

The literature on the physiology of reproduction not only shows the words fertility and fecundity used interchangeably, but the same is true of the terms fertilization and fecundation. Dalton (1875) and Flint (1876) employed fecundation as referring to the union of egg and sperm. Others as Draper (1856) and Dorland (1896) used either term when referring to this process, while later physiologists, as Landois and Sterling (1889), Howell (1901), Marshall (1910), Starling (1912) and Halliburton (1917) have given preference to the term fertilization. The similarity in the meanings of these two terms is further illustrated by their definitions as given by Dorland's Medical Dictionary (1919). Fecundation is defined as "Impregnation or fertilization," while fertilization is "the act of rendering fertile; fecundation." Furthermore, formal definitions of fertility and fecundity, as given, for example, by the Century Dictionary, treat them as practically

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synonymous. Fecundity is defined as "Fruitfulness; the quality of propagating abundantly; particularly the quality in female animals of producing young in great numbers." Turning to the definition of fertility we find: "The state of being fertile or fruitful; the quality of producing in abundance; fecundity; productiveness. . . ."

In common usage fertile is employed as the antithesis of sterile, that is an animal is said to be fertile when capable of producing offspring, while on the other hand a female is called fecund if she produces large numbers of offspring. The four terms fertility, fecundity, fertilization and fecundation refer then to one process, namely the production of offspring. But before offspring may result an egg and spermatozoon must have fused, and before this can occur ova must be liberated from the ovary. Ovulation occurs periodically and may continue to do so quite independently of the production of young. We have then two conditions, first, the discharge of eggs from the ovary and second the union of egg and sperm, normally resulting in the production of young. Pearl and Surface (1909, p. 81) in their work with fowls differentiated these two conditions, assigning the term fecundity to the former and fertility to the latter. Thus fecundity is "the innate potential reproductive capacity of the individual organism, as denoted by its ability to form and separate from the body mature germ cells. Fecundity in the female will depend upon the production of ova and in the male upon the production of spermatozoa." Fertility as defined by these authors is "the total actual reproductive capacity of *pairs* of organisms, male and female, as expressed by their ability when mated together to produce (i.e., bring to birth) individual offspring."

This definition of fecundity relates to *actual* fecundity, or the actual number of eggs discharged from the ovary of a female regardless of the production of offspring. Mention may be made of a second type of fecundity, namely *potential*, there being more potential ova present in the ovaries than can, because of physical limitation, be discharged in the lifetime of an individual. Poten-

tial fecundity is difficult, if not impossible, of measurement.<sup>2</sup> Actual fecundity is possible of determination in birds (except for resorbed eggs), but not in mammals, since the eggs of mammals are microscopic in size and hence are lost to record unless they undergo further development. Since, therefore, actual fecundity cannot be directly determined in mammals, it becomes necessary to express fecundity in them in terms of fertility as defined by Pearl and Surface. This may accordingly be designated as *observed fecundity*.

It would be of importance to determine, if possible, the degree of accuracy which may be obtained in expressing fecundity in this way. So far as the authors are aware there has been no direct attempt to make this determination. Certain embryological studies, however, supply observations which serve to give some idea of the degree of correspondence between actual and observed fecundity. For example, there are cases in which comparison may be made between the number of ripe follicles in the ovaries of any female and the average number of young normally produced in a single litter. Honoré (1900) in his work on the rabbit found five follicles about to rupture in one ovary of a female which had been bred a few hours previous to examination. No mention is made of the other ovary but, if we assume that as a matter of chance it had the same number of ripe follicles as the one examined, the total number in both ovaries would be ten, which is in excess of the average number of young per litter in rabbits, for according to Marshall (1910, pp. 588 and 591), who takes Spencer and Darwin as his authorities, six or seven is the average litter size in this species. A few similar data on the bat are available from the work of Van der Stricht (1901). An examination of the ovaries of several individuals revealed one, two or three ripe follicles. Taking Marshall (1910, p. 587)

<sup>2</sup> Pearl (1912) attempted to measure potential fecundity in the domestic fowl by counting the oocytes visible to the naked eye in the ovaries of several hens. Such counts, made for seventeen different individuals, showed each ovary to contain an average of 1676 oocytes. Pearl, however, states that this number is a minimum one, since there is a large number of oocytes invisible to the naked eye, but nevertheless present in the ovary and capable of further growth and development.

as authority again, the bat as a rule produces but one individual at a single parturition, so again there are more ripe follicles than offspring normally produced. These examples are inserted here merely to illustrate a possible way of studying actual fecundity in mammals, and as giving an indication that observed fecundity is not a complete measure of actual fecundity.

Other embryological studies make it possible to compare the number of corpora lutea with the number of embryos in the uterus. Sobotta (1896) in his studies on the mouse found three corpora lutea in one ovary of a female and three embryos in the corresponding horn of the uterus, while the other ovary showed two corpora lutea, but no mention is made of embryos in the horn of this side, so presumably there were none. Later the same author (1897) examined the ovaries of eight rabbits at different periods of gestation and found the following conditions. The largest number of corpora lutea in the ovaries of any one rabbit was twelve, with nine embryos in the uterus, while the smallest number of corpora lutea in any of the eight rabbits was four, with three embryos. In every case the corpora lutea exceeded the embryos in number. The total number of corpora lutea in all the rabbits was fifty-nine, an excess of eighteen over the total number of embryos, which was forty-one. Only 70 per cent, therefore, of the corpora lutea were represented by embryos.

There is still another possible method of comparison, namely, between the number of corpora lutea and the number of offspring normally produced by any female in a single litter. Data on this point are more extensive, but only the most important of these will be presented. Marshall (1904) in his study of the oestrous cycle in sheep examined the ovaries of fifty-five females. Forty-two individuals showed one ruptured follicle in one ovary or the other, twelve had two ruptured follicles, one in each ovary or both in one, while one individual had three ruptured follicles, two in one ovary and one in the other. Summarizing this Marshall says (p. 74): "Thus in less than 24 per cent of the cases examined where ovulation had occurred, was more than one follicle found to have discharged." He concludes: ". . . in view of the percentage of follicles discharged (as noted) being

scarcely, if at all, in excess of the usual percentage of lambs produced for the breeds in question, the converse of this statement is most probably also generally true." In other words about 76 per cent of the fifty-five ewes showed but one ruptured follicle, which indicates that practically three-fourths of these ewes would have produced but one offspring. According to Marshall this condition coincides with the average number of lambs actually produced singly by the breeds under consideration.\*

Corner (1915) in examining 128 pairs of ovaries taken from pregnant swine, found from one to sixteen corpora lutea in both ovaries, eight being the most common number. According to the author's records six was the most frequent number of pigs per litter, while the range was from one to ten. The number of corpora lutea accordingly exceeded the number of pigs per litter.

It is obvious from the foregoing embryological studies that there is some discrepancy between actual and observed fecundity. This may be accounted for in part by certain physiological conditions connected with ovulation and reproduction. For instance, fecundity in ovulating virgin females is undeterminable, but is presumably higher than in pregnant females, since ovulation rarely if ever occurs during pregnancy. Furthermore females may not be bred regularly, and often when they are bred they fail to conceive. The fact that the eggs discharged at any ovulating period may not all be fertilized has just been demonstrated. On the other hand eggs may be fertilized, attain some development, and then degenerate. In either case these eggs are lost to record. One of the causes of this degeneracy is genetic lethals, as illustrated by the death *in utero* of the homozygous yellow mouse (Ibsen and Steigleder 1917). Other points might perhaps be mentioned, but these will suffice to show that there are a number of factors causing discrepancies between actual fecundity in mammals and that which is observed.

\* Data presented by Bell (see p. 270 of this paper) and the present authors (p. 284) show the percentage of ewes producing single and twin lambs to be about equal, that is only 50 per cent of the ewes considered in these records drop single lambs.

In spite of these disturbing factors the discrepancy between observed and actual fecundity is not as great as might be supposed. This is of importance since the latter is the only practical measure of fecundity in mammals.

#### FECUNDITY AS AFFECTED BY AGE

Experiments and observations on multiparous animals have shown that when breeding is unrestricted the frequency of production of litters normally increases with the age of the female, at least up to a certain point. Records taken by Miss King (1916), for example, on the reproductive rates of seventy-six female rats revealed the fact that they produced fewer litters at the mean ages of 90 and 120 days than they did at 150, 180, 210 or 240 days. This point cannot readily be determined for the normally uniparous domesticated animals, in part because some of them ordinarily breed only at definite seasons, and even more because their breeding is not unrestricted, but is regulated by man to meet his convenience.

Records on multiparous animals further indicate that the average size of litter also increases with the age of the female until a fairly advanced age is attained. The data which show a similar physiological tendency in uniparous animals and man are rarely if ever interpreted as increase in litter size. This is because the occurrence of multiple births is relatively uncommon and hence in all cases the average litter size is only slightly in excess of one. This is particularly true in cattle and horses; in sheep, where the number of multiple births may be as great as 50 per cent, the average litter size may correspondingly be as high as 1.5, or even higher if any considerable proportion of the multiple births are triplets. Where the multiple births are practically all twin births, as in cattle, the "average litter size" is expressed by 1 plus the percent of multiple births. Average litter size for any age may readily be deduced from any expression of the proportion of multiple births to all births at that age.

In the following pages a review is first given of literature on the relation of age to fecundity in mammals, and this is followed by an analysis of data relating to cattle and sheep.



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## SUMMARY OF LITERATURE

Data relating to the subject of this paper pertain chiefly to domesticated rodents (rats, rabbits, and guinea-pigs), the larger farm animals (swine, sheep and cattle) and man. Records on the first class of animals have been taken directly from experiments; most of those on the second class from Herd Books; while the few on man are collected from hospital records.

Miss King (1916) draws the following conclusions from the breeding records of seventy-six rats: (1) “. . . a female rat reaches the height of her reproductive capacity when she is about seven months of age. This age represents also the median point in the animal's breeding career. That is one-half the total number of her offspring are produced by the time she has reached this age and one-half are produced afterwards” (p. 273). (2) The largest sized litters were produced by females of a mean age of 120 days. This was determined by averaging the litters produced by females of given ages. (3) The second litter is on the average the largest of the five or six usually produced by the rat. These last two points are practically the same in their significance, for, since rats produce their first litters at about a mean age of ninety days, they could not produce the second one till they were approximately 120 days old, the mean age at which the largest litters are cast.

Observations made by P. G. Bailey on the size of litters of rabbits are quoted by Hammond (1914, p. 266). The sizes of the litters are considered from the order of their occurrence, rather than the actual age of the mother. No mention is made

of the number of litters upon which the following figures are based. So far as they go, these findings show the second litter to be the largest, as in the rat.

<i>Litter sequence</i>	<i>Average size of litter</i>
First litter.....	5.58±0.32
Second litter.....	7.25±0.41
Third litter.....	7.08±0.38

Minot (1891) found that the number of guinea-pigs in a litter increases with the age of the mother up to 15½ months, when the greatest number of offspring are produced. Table 1, taken from Minot, shows in general an increase in litter size with the age of the female. The largest number of "first" litters cast contain one or two individuals and the largest number of "second" litters, three individuals. The suggestion is made that females might be kept in virginity for several years and then bred, in

TABLE 1  
*Sizes of successive litters in guinea-pigs (Minot)*

NUMBER OF LITTER	1	2	3	4	5	6	7	8.
First.....	16	25	9				1	
Second.....		9	13	6	1			
Third.....		6	6	3				
Fourth.....			2	2				

order to compare the size of their litters "with those of young primiperæ and of multiperæ of their own age."<sup>4</sup>

Marshall (1910, p. 590) makes a statement to the effect that dogs have fewer puppies in early litters than in later ones. The same is said to be true of the bear and elk, though no data are given to substantiate these statements.

More extensive observations relating to this subject have been made on swine and sheep than on any other farm animals, probably because of the importance of the matter to livestock breeders and the facility of obtaining data on these particular animals. Rommel (1907) has considered the relation of the

<sup>4</sup> An experiment to test this theory was started by Dr. H. L. Ibsen at this Station and is being continued by him at the Kansas State Agricultural College.

age of sows to the number of offspring produced. He found from a study of the Poland China Herd Books that the size of litters of sows increases with their ages as follows (p. 204):

<i>Age of sow</i>	<i>Average size of litter</i>
1 year.....	6.65
2 years.....	7.56
3 years.....	7.88
4 years.....	8.28
5 years.....	8.40

The above figures show an increase in litter size from one to five years. However five years cannot be definitely stated as the age for maximum litter-size production, because the data for subsequent ages were too few to be significant. The average size for all litters is 7.39.

Rommel points out that breeders should not dispose of their brood sows after producing one or two litters, but should keep them in order to obtain larger numbers of offspring.

Frölich and Georgs (1911) record the size of litter for a white breed of German swine (Edelschwein). Their results are obtained from the breeding histories of forty-six sows producing 1739 pigs. Since these authors consider the size of litters in relation to their sequence, rather than the actual age of the mother, the data which follow are not directly comparable to Rommel's and Minot's on swine and guinea-pigs.

<i>Litter number</i>	<i>Litter size</i>
1	7.98
2	8.74
3	8.26
4	7.72
5	7.54
6	9.00

Average for six litters.....8.22

The maximum size is said by the authors to be reached in the second litter, the large recorded number in the sixth litter being attributed to the fact that the less fertile sows have been eliminated, only the most fertile ones being retained. A comparison of this material with that of Rommel's reveals certain

differences. It is unreasonable to suppose that the sows reported by Rommel were only just producing their second litters at five years, and it is also improbable that the sows considered by Frölich and Georgs were five years old when they gave birth to their second litters. These discrepancies may be attributed in part at least to two factors: (1) Rommel's results were obtained from a larger number of sows than were those of Frölich and Georgs; (2) there may be breed differences.

Machens (1915) also records the sizes of litters produced by sows of various ages in another German breed of swine. His data, based on a study of 362 litters, do not agree with either those of Rommel or Frölich and Georgs, since Machens finds the fourth litter the largest produced by this breed of sows. The average sized litter for sows of all ages is 9.56, which is higher than the averages for the other two breeds of swine just discussed.

In 1909 Mumford (1917) started an experiment with swine, which bears directly on the present subject. He obtained litters from immature sows (four or five months old), half mature sows (eighteen months old) and mature sows (twenty-four to thirty months). The results up to 1913 showed the average size of the litters for the three classes as follows, immature 4.8, half mature 6.3 and mature 6.5.

In the twenty-eighth annual Report of the State College of Washington (1919) data are presented on the average size of litter for sows of various ages as follows: 16 one year olds 6.12, 17 two year olds 7.7, 10 three year olds 7.8, and 4 four year olds 7.9. Records on older females are not given.

Hammond (1914) examined the ovaries of eighteen young sows, killed just after the heat period, and found the average number of corpora lutea in both ovaries to be  $14.3 \pm 0.39$ , while the ovaries of nine older sows showed  $19.77 \pm 1.26$  corpora lutea.

The foregoing results on swine differ somewhat in details, but indicate that in general older sows tend to produce more eggs than younger ones, and consequently larger litters. (Note page 290.)

Heape (1899) found that in the Dorset Horned breed of sheep fewer twins were dropped by younger than older females. Only a bare statement is made, no data being presented.

Carlyle and McConnell (1902) show from records taken on the flock at the Wisconsin Station, that the percent of twin and triplet births increases with the age of the ewe in mutton breeds. The largest percentages of single, twin and triplet births are produced respectively by two, five and six year old ewes. Later data, reported by Humphrey and Kleinheinz (1907) from the same flock, confirm the findings of Carlyle and McConnell as to the ages at which ewes produce the largest percent of single and triplet births, but they found that the highest percent of twin births was produced by four year old instead of five year old ewes.

Pearl (1913) gives the unusual breeding record of a single ewe for nineteen years. She produced single lambs at one and two years of age, twins at three years, from four to nine years triplets for each year, twins again for each year from her tenth to fifteenth and singles for her sixteenth and seventeenth years. The maximum fecundity occurs at 7.34 years. This single case illustrates both the increase and subsequent decline in litter size with the advancing age of the female.

Alexander Bell (1904-1912) in his experiments with multi-nippled sheep did not find them, as he expected, more fertile than their normally nipped sisters. However, in his 1912 report Bell concludes that all the older ewes had a greater tendency to produce multiple births than the younger females. Thirty-six per cent, apparently, of all lambs born of young ewes were twins, while 60 per cent of the lambs born in the spring of 1912 of three year old ewes were twins. Popenoe (1914) quotes from another paper of Bell's, "ewes four, five and six years old yield a larger percentage of twins than younger or older ewes." These data of Bell's and the preceding findings on sheep lead to the same general conclusion as did those on swine, namely, that the tendency to produce larger litters is greater among older than younger females and may be followed by a subsequent decline in animals kept to a greater age.

Data on the frequency of multiple births in cattle are meager, though a great many items on the reproductive records of individual cows might be gleaned from the stock journals. The

authenticity of these scattered notes may in some cases be questioned. Nevertheless we will present three cases as given by Pearl (1912). The first, taken from the National Livestock Journal (1879), states that a certain cow gave birth to twins

TABLE 2  
*Breeding history of cow owned by Mr. Starret (Pearl)*

YEAR	AGE	NUMBER OF CALVES PRODUCED
Born		
1900		
1902	2	1
1903	3	1
1904	4	1
1905	5	2
1906	6	2
1907	7	3
1909	9	1
1910	10	3
Total .....		14

TABLE 3  
*Breeding history of cow cited by McGillivray (Pearl)*

YEAR	AGE	NUMBER OF CALVES PRODUCED	REMARKS
Probably born 1840			
1842	2	1	This was the cow's first calf
1843	3	3	All lived to be adults
1843	3	4	One died (7 calves in one year)
1844	4	2	Lived to maturity
1845	5	3	Lived to maturity
1846	6	6	All died prematurely
1847	7	2	Lived to maturity
1848	8	4	
Total .....		25	

three times in succession and then to triplets. Pearl next gives the breeding history of a cow belonging to a Mr. Starrett living near Waldoboro, Maine. This history is reproduced in table 2. It shows that the cow reached the high point of her reproductive

activity at seven years, when she produced triplets. The fact that she produced triplets again at ten years would seem to indicate that the high fecundity was being maintained at least to that age. A third case (table 3) presented by Pearl is taken from McGillivray's "Manual of Veterinary Science" (1857). This shows a more or less regular increase in the number of young produced by a cow up to the age of six years, when she is claimed to have aborted six embryos.

The ages at which various women produced 756 pairs of twins as reported by Duncan (1866) are shown in table 4. The records

TABLE 4  
*Occurrence of twins in 1512 parturient women (Duncan)*

	AGES							Total
	15-19	20-24	25-29	30-34	35-39	40-44	45	
Collins.....	3	53	76	71	28	8	1	240
M'Clintock.....	2	23	45	41	17	1		129
M'Clintock and Hardy.....	1	20	34	26	12	2		95
Chiari Braun and Spaeth.....	1	25	36	26	4	2		94
Statistics of 1855.....	3	28	46	58	52	11		198
Total.....	10	149	237	222	113	24	1	756

were collected by the author from various sources and recorded under the ages of the mothers, which are arranged in five year periods. As Duncan (p. 73) says: "This table shows that in the general population it so happens that the number of twins born increases with the age of the mother until the age from twenty-five to twenty-nine inclusive is reached and that after this age is passed the number of twins born regularly diminishes." Duncan quotes from Dr. Collins to the effect that the mean age of 16,385 parturient women is twenty-seven, while that for 240 women bearing twins is twenty-nine.

## MULTIPLE BIRTHS IN CATTLE

*Source of data*

In undertaking the present study the American Herd Books of the two beef breeds, Hereford and Aberdeen-Angus, seemed to offer the largest amount of desirable data. In the first place beef breeds were chosen instead of dairy because most breeders of dairy cattle do not register females twinned with bulls, as they are so commonly sterile, but as milk production is of little consequence to beef breeders they do not discriminate so closely against these individuals. The total number of twins actually produced is therefore, more nearly represented by the beef registries than by the dairy. Second, these particular breeds were chosen instead of the Shorthorn or Red Polled, because the manner of recording animals is much the same throughout both these registries and the entries are made in such a way as to facilitate the compiling of data for our purposes.

The records used were taken from the first forty-two volumes of the American Hereford Record and the first twenty-six volumes of the American Aberdeen-Angus Association. The former contain the registries of 536,000 individuals and cover a period of years from 1880 to 1916, while the latter contain the entries of 220,500 animals from 1886 to 1916.

The details of entry for each animal vary somewhat in the two breeds, but in general they apply to both sets of Herd Books. The details are as follows: registration number, sex, name of animal, date of its birth, names and numbers of the sire and dam, and names of owner and breeder. If any individual is a twin, triplet, or quadruplet this fact is inserted below the animal's name.

*Tabulation and treatment of records*

Tabulations were made in order to obtain data on (1) the number of multiple births recorded and (2) the ages of the dams at the time of these births. For (1) records of all individuals designated twin, triplet or quadruplet were assembled from the sixty-eight volumes of the two registries. For (2) it was, first,



necessary to note the dates of birth of all twins, triplets and quadruplets and also the registry numbers of their dams. Then by turning to the latter the dates of birth of the dams were procured. Finally a determination of the interval between the dates of birth of the various twins, triplets, and quadruplets and the dates of birth of their respective dams gave the ages of these dams at the time of calving. These results were next arranged according to the various ages of the dams.

The operations of assembling the data and making the necessary age determinations were independently checked. It was now felt that these data were as reliable as possible, considering that they were based on Herd Book records, in which a small percentage of errors is likely to occur. These may be due to mistakes in reporting, to clerical and typographical inaccuracies, and even to occasional deliberate falsification of facts by breeders. However, since the numbers dealt with here are large, the effect of this small percentage of error is negligible.

The above determinations gave the actual numbers of cows producing twins, triplets and quadruplets at different ages. These numbers are based on records taken from cattle in which the rate of production, record of production, and to a large extent the mortality are controlled by man. That is, the present data deal with a selected population of animals and not with a natural one. It was therefore necessary to consider the number of multiple births occurring among cows of a given age in this selected population, in proportion to the total number of births occurring among all dams of the same ages. In order to do this the ages of all cows at the time of calving were calculated. But since it would have been an enormous task to have made this calculation for the dams of the 750,500 animals recorded, the determinations were made only for every one-hundredth individual registered, such a selection being considered a fair random sample of the entire population. As there were a few cases in which all the desired information was not given, the ages at calving were actually obtained for 7471 cows instead of 7505. The number of dams in each age group was now multiplied by 100 to represent the total population.

The data on multiple births and all births were next tabulated according to the various age groups of the dams and the above-mentioned proportions were obtained. The number of multiple births occurring among cows of a given age per one thousand cows calving at the same age was calculated, the ratio obtained from using a thousand as a basis giving the most convenient numbers for final comparison. Although the general term multiple birth has been employed in the above discussion, as a matter of fact twin births only were taken into consideration in these calculations, the numbers of triplets and quadruplets being too few to make any observable difference in the final results. There were but seven of the former and one of the latter registered in both sets of Herd Books.

*Records from Hereford Herd Books*

Table 5 shows the results of the compilation of the data on the Hereford breed. The first column gives the ages of the dams from one to thirty-three years and the second column the actual number of recorded multiple births for dams of each age. The data in column three represent the total number of cows calving or the total number of all births occurring at each age, the actual figures obtained having been multiplied by one hundred, as discussed in the previous section. Column four gives the number of multiple births per one thousand cows calving at the various ages.

The graphs in figure 1 are based on table 5. Curve 1H, constructed from the data in column two (total number of multiple births recorded), shows a fairly rapid ascent from one to four years and is almost as high at five years, after which it descends very gradually. Graph 2H, representing the total number of cows calving at each age, is similar in general form to 1H, the number of records included here, however, being much larger and the mode falling at three instead of four years. The descent of these curves after three and four years is due to the general tendency among breeders to dispose of older cows, unless they are especially valuable, in order to make way for the younger

ones. As a consequence there are fewer data for older cows. That this is the cause of the low number of multiple births recorded for dams beyond four years is shown by graph 3H, based on column four of the table. These comparative data show a consistent rise in the relative frequency of multiple births

TABLE 5  
*Data from Hereford Herd books, volumes 1-42*

AGE	NUMBER OF MULTIPLE BIRTHS	TOTAL NUMBER OF COWS CALVING	NUMBER OF TWINS PER 1000 COWS CALVING
1	3	3,000	1.00
2	120	71,800	1.67
3	281	89,300	3.14
4	351	79,800	4.39
5	346	64,700	5.34
6	288	53,700	5.36
7	248	44,300	5.59
8	205	35,400	5.79
9	161	26,700	6.02
10	127	20,100	6.31
11	95	14,000	6.78
12	67	9,700	6.90
13	45	6,500	6.92
14	26	3,900	6.66
15	10	2,100	4.76
16	7	1,200	5.83
17	4	600	6.66
18		400	
19	1	200	5.00
21		200	
22		100	
23		100	
29	2		
33		100	
Blanks	42	8,100	
Total.....	2,429	536,000	
Minus blanks.....	42	8,100	
Total.....	2,387	527,900	4.52

up to twelve or thirteen years of age. Therefore the age for highest multiple birth production is not at four but at least as late as twelve or thirteen years. Beyond this age the data are so few that the curve is irregular and has no significance.

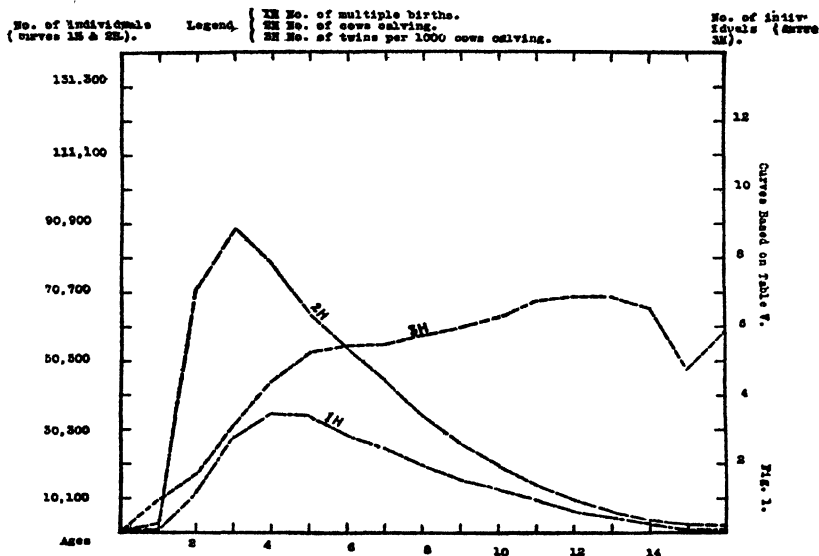


FIG. 1

*Records from Aberdeen Angus Herd Books*

Data on the Aberdeen-Angus breed, corresponding in material and arrangement to that in table 5, are incorporated in table 6. The total number of records available on the Angus are only about half as many as on the Hereford. Furthermore, the oldest dam reported as calving in this breed was aged twenty-five years, while in the Hereford, records were given for dams up to thirty-three years of age.

Figure 2, based on table 6, is in general similar to figure 1. Curve 1A represents no striking difference from 1H, as the degree of ascent and descent of both graphs is practically the same and the modes of both curves fall at four years. The age at which the largest number of multiple births occurs is accordingly the same for both breeds. Curves 2A and 2H are also similar in general appearance, but the mode of the former occurs at two instead of three years, showing that the largest number of recorded births occurs a year younger for Angus dams than for

Hereford. This means that Angus heifers are in general bred at an earlier age than Herefords, which is a natural sequence of the characteristic tendency of the Angus breed to early maturity.

TABLE 6  
*Data from Aberdeen-Angus Herd books, volumes 1-26*

AGE	NUMBER OF MULTIPLE BIRTHS	TOTAL NUMBER OF COWS CALVING	NUMBER OF TWINS PER 1000 COWS CALVING
1	3	5,800	0.51
2	62	34,300	1.80
3	111	32,800	3.38
4	144	31,200	4.61
5	118	25,000	4.72
6	116	21,300	5.44
7	74	15,900	4.65
8	67	15,200	4.40
9	57	11,200	5.08
10	50	8,500	5.88
11	22	6,200	3.54
12	27	3,700	7.29
13	22	3,800	5.78
14	6	1,900	3.15
15	8	600	13.33
16	5	1,000	5.00
17	2	300	6.66
18	3	300	10.00
19	1	100	10.00
21	2		
22		100	
25	2		
Blanks	25	1,300	
Total.....	927	220,500	
Minus blanks.....	25	1,300	
Total.....	902	219,200	4.11

Curve 3A showing the number of twins per thousand total births in the Angus, resembles very closely the corresponding curve (3H) for the Herefords, but is more irregular beyond nine or ten years owing to the relatively small number of records.

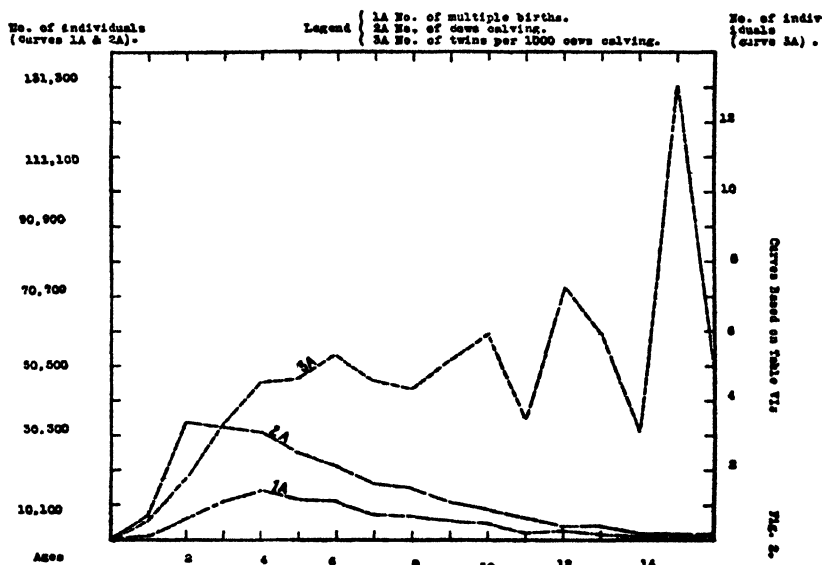


FIG. 2

*Combined records of both breeds*

Figure 3 is constructed from the data in table 7 made by combining the records of tables 5 and 6. The curves of this figure are the same in general form as the corresponding curves in the two preceding figures, but are smoother because of the larger amount of data upon which they are based. This emphasizes again the fact that the two sets of data, from the two different breeds, exhibit essentially the same features. The significant characteristic of curve 3C is its consistent rise to at least ten or twelve years and, though the data are relatively few and the curve consequently less regular beyond this point, its inclination to maintain about the same level, as may be seen from the data in column four of table 7. At any rate, there is no indication of a decisive falling off in the tendency to produce twins.<sup>5</sup>

<sup>5</sup> Dr. Florence E. Allen fitted curves to the number of cows calving and number of multiple births, as shown in figure 3, according to Pearson's methods given in the Philosophical Transactions, Pt. 1, A, 1895. The curve was fitted to the number of multiple births per 1000 cows calving by taking the ratio of the formulae of the curves 1C and 2C.

TABLE 7  
*Combined data on both sets of herd books*

AGE	NUMBER OF MULTIPLE BIRTHS	TOTAL NUMBER OF COWS CALVING	NUMBER OF TWINS PER 1000 COWS CALVING
1	6	8,800	0.68
2	182	106,100	1.71
3	392	122,100	3.21
4	495	111,000	4.45
5	464	89,700	5.17
6	404	75,000	5.38
7	322	60,200	5.34
8	272	50,600	5.37
9	218	37,900	5.75
10	177	28,600	6.18
11	117	20,200	5.79
12	94	13,400	7.01
13	67	10,300	6.50
14	32	5,800	5.51
15	18	2,700	6.66
16	12	2,200	5.45
17	6	900	6.66
18	3	700	4.28
19	2	300	6.66
21	2	200	10.00
22		200	
23		100	
25	2		
29	2		
33		100	
Blanks	67	9,400	
Total.....	3,356	756,500	
Minus blanks....	67	9,400	
Total.....	3.289	747,100	4.40

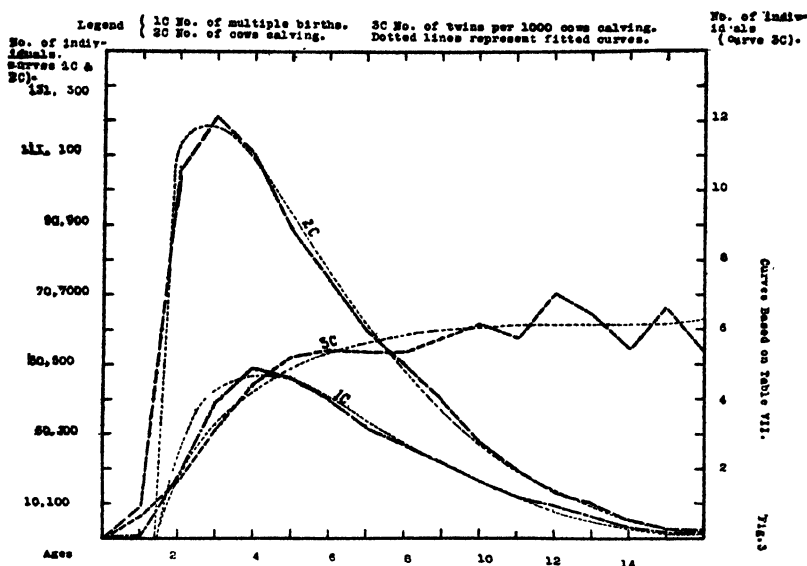


FIG. 3

*Triplets and quadruplets in both breeds*

As already mentioned only twins were considered in the preceding tables, the numbers of triplets and quadruplets being too few to have an appreciable influence on the results. The recorded triplets and quadruplets, shown in table 8 are given only for the interest attached to the occurrence of more than one or two calves at a single parturition.

A consideration of the frequency of the different orders of multiple births shows that there is one twin birth to every 221 births in the Hereford breed and one to every 243 in the Aberdeen-Angus. For the two breeds combined the twin births are one in 224, or 0.4 per cent of all births. This is considerably lower than Newman's statement (1917, p. 122) that twins in cattle constitute about 2 per cent of the total number of births. The percentage of twinning in man, calculated from the ratio given by Bland (1781) and Duncan (1866), who state that twin births occur once in every 80 deliveries, is 1.25 per cent. The



frequency is greater in sheep, as shown by Plumb's data (1905) taken from the Shropshire Flock Books. The number of twins recorded in a population of 23,037 individuals is 9053. Since, however, it is not stated when both individuals of a set are included and in how many cases only one is recorded, it is impossible to tell just how many births these 9053 recorded twins represent. Obviously it could not be more than 9053 nor less

TABLE 8  
*Triplets and quadruplets in both sets of herd books*

BREED	AGE OF DAM	NUMBER OF CASES	
		Triplets	Quadruplets
	<i>years</i>		
Hereford.....	2	0	1
	4	2	0
	7	2	0
	10	1	0
Angus.....	4	2	0
Total.....	Av. 3.86	7	1

than half that number; in other words the twin births were at least 19.6 per cent of the total births, but not more than 39.2 per cent.<sup>6</sup> The frequency of triplets, as found in the present study of cattle, is as follows: one in every 105,580 births in the Hereford; one in every 109,600 in the Angus; or one in every 106,728 births in the two combined. The percentage of triplets

<sup>6</sup> This uncertainty does not exist in our cattle data, as the records were carefully checked to determine just which recorded twins belonged in sets, so that in this way the actual number of twin births involved was ascertained.

Equation for cows calving, origin at the mode (2C).

$$y = 1212.34 \left( 1 + \frac{x}{1.27648} \right)^{0.29088} \left( 1 - \frac{x}{21.42753} \right)^{4.88284}$$

Equation for twins, origin at the mode (1C).

$$y = 468.45 \left( 1 + \frac{x}{2.78} \right)^{0.8369} \left( 1 - \frac{x}{22.7117} \right)^{6.8348}$$

and quadruplets in all multiple births is 0.24 per cent, which is decidedly lower than figures found by Cole (1916), who reported seven cases of triplets in 303 multiple births. The true percentage, however, probably is between these two figures, as the herd books do not include all triplets dropped, as for example those which are aborted, while Cole's data were collected without regard to the proportions of the different classes of multiple births and probably include an undue number of triplet records. Only one quadruplet was recorded for the Hereford and none for the Aberdeen-Angus. There were no records on larger numbers of calves dropped at one time, though cows have been reported as giving birth to as many as six fetuses (p. 272).

#### MULTIPLE BIRTHS IN SHEEP

As noted in the Summary of Literature (p. 270) the first data collected by Carlyle and McConnel (1902) from the flock records of the University of Wisconsin indicated that the highest percent of twin births in sheep occurred among five year old ewes, while the later report of Humphrey and Kleinheinz (1907) gave four years as the age at which the highest percent of twins was found. Later records from the same flock have been compiled by the present authors and are combined with the earlier ones in table 9, the arrangement of the data being essentially the same as in the preceding tables of multiple births in cattle, except that twins and triplets are first considered separately and then combined in column four. Twins and triplets were combined in sheep because of the relatively large number of triplets in sheep as compared with cattle. The number of triplets is not so large, however, as to influence the final calculations in any marked degree. This is shown by the frequency distributions in columns 6 and 8, which are essentially similar. Although the present records are considered on a per thousand basis instead of percentage, as was done in the two earlier reports on the same flock, they admit of ready comparison with the latter by a decimal shift of one place.

Figure 4 is based on data in table 9. The graph for the total number of ewes lambing at the different ages (column 5) begins at the highest point, two years, after which there is a consistent and regular decline. The curve for the total number of multiple births (column 4) is somewhat similar, except that the highest point is reached at three years and the drop is slightly more gradual. These two curves merely show that more records are available on two and three year old ewes than on older ones, because of the general custom among sheep raisers to dispose of older individuals. The horizontal dotted line shows the number

TABLE 9  
*Data from University of Wisconsin flock, 1890-1917*

AGE	TWINS	TRIPLETS	TOTAL NUMBER OF MULTIPLE BIRTHS	ALL BIRTHS	TWINS PER 1000 BIRTHS	TRIPLETS PER 1000 BIRTHS	MULTIPLE BIRTHS PER 1000 BIRTHS
2	159	4	163	367	433.24	10.89	444.14
3	156	12	168	284	549.29	42.25	591.54
4	136	13	149	223	609.86	58.29	668.16
5	105	7	112	162	648.14	43.20	691.35
6	54	9	63	93	580.64	96.77	677.41
7	26	2	28	48	541.66	41.66	583.33
8	10	1	11	15	666.66	66.66	733.33
9	2		2	2	1000.00		1000.00
All ages....	648	48	696	1194	542.71	40.20	582.91

of multiple births per one thousand births produced by ewes of all ages or, in other words, the average age at which multiple births occur. The curves representing the number of multiple births per one thousand ewes lambing at each age (twins and triplets combined) is the significant one for our consideration. This shows that according to the present records the greatest number of multiple births is produced by five year old ewes. The four and six year old females also show a high degree of fecundity. After six years, however, there is a sudden drop, which is probably significant since the number of records for seven years is comparatively large. The rise at eight years

may be due to lack of sufficient data for that age and at nine years it is obviously due to this cause, since it is not at all probable that all nine year old ewes would produce twins.

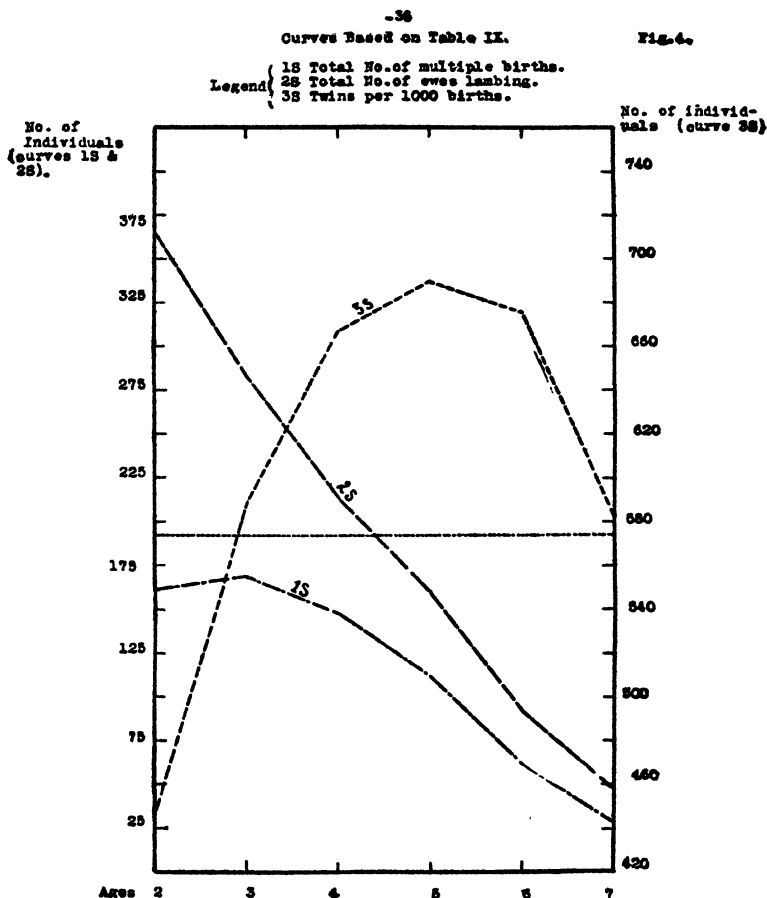


FIG. 4

The combined data on forty-eight sets of triplets are given in column three. As shown in column seven, six year old ewes produce the largest number of triplet sets per thousand births.

## DISCUSSION

Observations on fecundity as affected by the age of the female in uniparous animals coincide with similar ones on multiparous animals. All of the instances cited lead to the same general conclusion, namely, that size of litter increases as the female advances in age. In fact, so far as we are aware, there is no contradictory evidence. Some of the records, however, show a decline in litter size after the female has reached a fairly advanced age, while other data fail to show such a regression. This condition, nevertheless, may be attributed to lack of data on individuals of sufficiently advanced ages to indicate such a decline and to the variation in the span of the reproductive periods of the different breeds of animals. For example, some cases relating to swine showed a continued increase in litter size, while others, in which the data were fuller for sows of advanced years, indicated a decline. A comparison of the sheep and cattle records may illustrate the second point in regard to length of reproductive periods. The cattle records, so far as they go, show no decrease in litter size but indicate a comparatively long reproductive period. The sheep data on the other hand show a decrease in litter size and also a shorter reproductive period. This longer reproductive period in cattle combined with the general tendency among breeders toward early disposal of their stock reduces the number of older cows, which might calve in their later years, and hence, the lack of records on such individuals. Although most sheep are also slaughtered at comparatively early ages, the fact that their reproductive period is shorter makes more data available.

This discussion is presented as a possible explanation for the lack of a decrease in litter size in the present cattle data.

## SUMMARY

1. The terms fecundity and fertility (and similarly fecundation and fertilization) have commonly been used interchangeably.
2. Fecundity in the female may be *potential* (the sum total of ova capable of being produced by the ovary), *actual* (the ova

actually matured and discharged) and *observed* (the ova of which there is visible evidence, as by the production of eggs or young). The ability to produce offspring is defined by Pearl and Surface as *fertility*, and in mammals this is the same as observed fecundity.

3. Embryological evidence from a number of forms indicates that, in most if not in all cases, actual fecundity is somewhat greater than the observed. The discrepancy is probably not so great, however, but what the number of offspring produced make a fair measure of fecundity.

4. Observations on multiparous animals (rats) show that the frequency of production of litters increases with the age of the female, at least within limits. This point is not capable of determination in the uniparous domesticated animals, at least on the data available.

5. The evidence also indicates, that in animals in general, there is an increase of litter size with the increasing age of the male. This usually goes to a maximum beyond which there is a decline. In the uniparous forms litter size is more commonly expressed in terms of the relative production of multiple births to all births.

6. A review of the literature shows more or less fragmentary records on a considerable variety of animals, which all tend to substantiate the above statements.

7. The data on age of dams at time of giving birth to the 747,100 individuals recorded in the American Hereford and Aberdeen-Angus Herdbooks, volumes 1-42 and 1-26 respectively, show that there is at first a relatively rapid increase in fecundity with the advancing age of the female, followed by a more gradual rise, and without indication of a subsequent decline.

8. From the records of age of dam at birth of 1194 lambs in the flock of the University of Wisconsin, a similar curve was obtained, except that in this case it reached its maximum for dams at five years of age, beyond which there was a decided drop.

9. These findings on cattle and sheep coincide with earlier ones on other animals, since all show a general tendency for older females to produce larger litters than younger ones.

## REFERENCES

- BELL, ALEXANDER GRAHAM 1904 The Multi-nippled sheep of Beinn Bhreagh. *Science N. S.*, xix, no. 489, May 13, 767-768.  
1912 Sheep breeding experiments on Beinn Bhreagh. *Science N. S.*, xxxvi, no. 925, September 20, 378-384.
- BLAND, ROBERT M. D. 1781 Some calculations of the number of accidents and deaths which happen in consequence of parturition. *Philosophical Transactions of the Royal Society of London*, lxxi, part 1, 355-371; 2 woodcuts.
- BONNEVIE, KRISTINE 1919 Heredity in twins. *Eugenical News*, iv, no. 11. November, 92.
- CARLYLE, W. L., AND MCCONNELL, T. F. 1902 Some observations on sheep breeding from the experiment station flock records. *University of Wisconsin Agricultural Experiment Station. Bulletin*, no. 95, 1-19.
- COLE, LEON J. 1916 Twinning in cattle with special reference to the free martin. *Science N. S.*, xliii, no. 1101, February 4, 177-178.
- CORNER, GEORGE W. 1915 The corpus luteum of pregnancy, as it is in swine. *Carnegie Publication* 222, no. 5, ii, 71-94, 3 plates.
- DALTON, J. C. 1875 A Treatise on Human Physiology. 6th Edition. Pp. xxiv + 825. Henry C. Lea.
- DORLAND, W. A. N. 1896 A Manual of Obstetrics. Pp. 7-760. W. B. Saunders Co.  
1919 The American Illustrated Medical Dictionary. 10th edition. Pp. 15-1201. W. B. Saunders Co.
- DRAPER, J. W. 1856 Human Physiology Statistical and Dynamical, or Conditions and Causes of the Life of Man. Pp. xvi + 649. Harper & Bros.
- DUNCAN, J. MATHEWS 1866 Fecundity, Fertility, Sterility and Allied Topics. Pp. xvi + 378. Adam and Charles Black, Edinburgh, Scotland.
- FLINT, AUSTIN 1876 A Text Book of Human Physiology. Pp. xviii + 978. D. Appleton & Co.
- FRÖHLICH UND GEORGS 1911 Fruchtbarkeit und Geschlechtsverhältnis beim weissen Edelschwein. *Jahrbuch für wissenschaftliche und praktische Tierzucht*. 6. Jahrg., S. 451-454. Hannover.
- HALLIBURTON, W. D. 1917 Handbook of Physiology. 13th edition. Pp. xx + 930. P. Blakiston Sons & Co.
- HAMMOND, JOHN 1914 On some factors controlling fertility in domestic animals. *Journal of Agricultural Science*, vi, pt. 3, 263-277, 1 plate.
- HEAP, H. 1899 Fertility in sheep. *Journal of the Royal Agricultural Society of England*. 3rd series. 10, pt. 2, 217-248.
- HONORÉ, CHARLES 1900 Recherches sur l'ovaire du lapin. I. Note sur les corps de Call et Exner et la formation du liquor folliculi. *Archives de Biologie*, xvi, 537-559.
- HOWELL, W. H. 1901 An American Test Book of Physiology. Vol. II, 2nd Edition, pp. 11-553. W. B. Saunders Co.
- HUMPHREY, GEO. C., AND KLEINHEINZ, FRANK 1907 Observations on sheep breeding from records of the university flock. 24th Annual Report of Wisconsin Agricultural Experiment Station, 25-40.

- IBSEN, H. L., AND STEIGLEDER, E. 1917 Evidence for death in utero of the homozygous yellow mouse. *American Naturalist*, li, December, 740-752.
- KING, HELEN DEAN 1916 The relation of age to fertility in the rat. *Anatomical Record*, xi, no. 5, December, 269-287.
- LANDOIS, L., AND STERLING, WM. 1889 A Text-Book of Human Physiology Including Histology and Microscopic Anatomy, etc. Pp. xxxii+974. P. Blakiston Sons & Co.
- MACHENS, DR. A. 1915 Fruchtbarkeit und Geschlechtsverhältniss beim veredelten Landschwein. *Berliner Tierärztliche Wochenschrift*, 31 Jahrg., no. 47, 25 Nov., 559-562.
- MARSHALL, F. H. A. 1904 Formation of corpus luteum in the sheep. *Philosophical Transactions of the Royal Society of London*. B, cxevi, 47-97, plates 7-10.  
1910 The Physiology of Reproduction. Pp. xvii+706. Longmans, Green & Co.
- MINOT, CHARLES SEDGWICK 1891 Senescence and rejuvenation. First Paper—On the weight of guinea pigs. *Journal of Physiology*. xii, 97-153, plates II, III, IV.
- MUMFORD, F. B. 1917 The Breeding of Animals. Pp. xvii+310. Macmillan Co.
- NEWMAN, H. H. 1917 The Biology of Twins. Pp. ix+185. University of Chicago Press.
- PEARL, R., AND SURFACE, FRANK M. 1909. II. Data on the inheritance of fecundity obtained from the records of production of the daughters of "200-egg" hens. *Maine bulletin* 166, March, 49-84.
- PEARL, R. 1912 The mode of inheritance of fecundity in the domestic fowl. *Journal of Experimental Zoology*, xiii, no. 2, August, 153-268.  
1912 Triplets in calves. *Maine bulletin* 204. September, 259-282.  
1913 Note regarding the relation of age to fecundity. *Science N.S.*, xxxvii, no. 945, February 7, 226-228.
- PLUMB, C. S. 1905 Prolificacy with ewes and breeds of sheep. *Proceedings of the 26th Annual Meeting of the Society for the Promotion of Agricultural Science*, 99-103.
- POPEOE, P. 1914 Sex determination in sheep. Compiled by the Editor, from the Records of Alexander Graham Bell. *Journal of Heredity*, v, no. 2, February, 47-57.
- ROMMEL, G. N. 1907 Inheritance of litter size in Poland China sows. *American Breeders' Report*, iii, 201-208.
- SOBOTTA, J. 1896 Über die Bildung des Corpus Luteum bei der Maus. *Archiv für Mikroskopische Anatomie*, xlvii, 261-308.  
1897 Über die Bildung des Corpus Luteum beim Kaninchen. *Anatomische Hefte*. Vol. 8; pp. 472-521.
- STARLING, E. H. 1912 Principles of Human Physiology. Pp. xii+1423. Lea and Febiger.
- STREBEL 1909 The fitness of twins in cattle and sheep for breeding. *Deutsche Landwirts Presse*, xxxvi, no. 84, 897-898; *E. S. R.*, 1910, xxii, 570.



VAN DER STRICHT, DR. O. 1901 La rupture du follicule ovarique et l'histogénèse du corps jaune. Comptes Rendues de l'Association des Anatomistes. 3 Lyon; pp. 33-41.

28th Annual Report of the Agricultural Experiment Station of the State College of Washington. 1919 Relation of breed and age of service to prolificacy. Bulletin 153. January; 9-10.

NOTE: Illinois Bulletin No. 226 (May, 1920) on Variations in Farrow; with Special Reference to the Weight of Pigs, by W. J. Carmichael and John B. Rice, has come into our hands since the present paper went to press. The data consists of records on 708 litters totaling 5774 pigs. The general average litter size is accordingly 8.1. The authors state (p. 72) that there was "a gradual increase in the size of the litter as the sows grew older up to the time they were three years old," after which age the tendency for decrease in litter size became apparent. The litter sizes of sows of one and one and a half years old are 7.5 and for two year olds or over 8.6.

# AN UNUSUAL OUTBREAK OF ROPY MILK

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## INTRODUCTION

The slimy or ropy fermentation is the abnormal change in milk that is most frequently reported to the dairy section of the Iowa agricultural experiment station. A considerable number of outbreaks of this kind have been studied from the standpoint of the causative organism<sup>1</sup> and *Bact. viscosum* has been isolated more than any other type. Recently an organism not previously encountered has been found as the cause of ropy milk and the results obtained in its study are given below.

## HISTORY OF THE TROUBLE

An outbreak of ropy milk was reported to the dairy section from one of the smaller towns in the state. The ropy condition was observed only in the supply of one dealer and seemed to be present mainly in the cream, the usual complaint being that, when the cap was removed, cream adhered to it and was drawn out into strings several inches long. Two samples were sent to the laboratory for examination with the following designations:

*Sample 1.* Ropy cream.

*Sample 2.* From same dairy as sample 1 two days later. Shows but little "ropy."

It was necessary to send the samples without ice and they arrived at the laboratory in a sour condition. Transfers made into litmus milk and held at room temperature coagulated rapidly without any development of ropiness, while others maintained at 10°C. showed nothing but a reduction of the litmus even after several days. Infusion agar plates, poured with the original

<sup>1</sup>See Buchanan and Hammer, Res. Bul. 22. Iowa Agr. Expt. Sta. 1915, for a review of the literature on ropy milk and an account of some of the earlier outbreaks studied at the Iowa Station.

material as soon as it arrived, and incubated at 20°C. showed after two days a considerable number of viscous colonies that somewhat resembled colonies of *Bact. viscosum* but which, when older, were considerably more opaque than colonies of this organism. Sample 1 showed a much larger percentage of viscous colonies than did sample 2. Litmus milk tubes inoculated with some of the viscous colonies and held over night at room temperature showed an extremely ropy condition without any evident change in the reaction of the milk. With an increase of twenty-four hours in the age of these cultures there was, however, a pronounced digestion of the milk and it seemed evident that the organism was not the usual type (*Bact. viscosum*) causing ropiness in Iowa. This idea was substantiated by a microscopic examination which showed that the organism was a Gram + coccus.

Shortly after the above mentioned outbreak was reported, a letter, telling of trouble from ropy milk, and a sample were received from a producer located almost 100 miles from the city in which the outbreak had occurred. The sample was not ropy and still sweet on arrival and was plated on infusion agar and the plates incubated at 20°C. Viscous colonies quickly developed and these when inoculated into litmus milk and held at room temperature produced a ropy condition without any noticeable change in the reaction and, later, an extensive digestion of the milk. A microscopic examination showed the organism to be a Gram + coccus, and its similarity to the organism isolated from the outbreak referred to above is thus evident.

The isolation from two widely separated localities of ropy milk organisms that a preliminary examination showed to be the same seemed especially important in view of the fact that this type had not previously been encountered in a considerable number of outbreaks of ropy milk that had been investigated. Inquiry showed, however, that the milk causing the outbreak was shipped in by the dairymen sending in the sample from the second source, and the relationship between the two complaints was thus cleared up.

The unusual character of the organism suggested an attempt to determine its source. Although at the time such an attempt was made ropiness had not been observed for some time, the producer collected samples in various ways in sterile bottles sent from the laboratory. The plates poured from the samples on arrival and incubated at room temperature did not show viscous colonies, although the number of colonies per plate was low. The original samples failed to develop a ropy condition when held at room temperature. After the samples had been at room temperature for about two days they were plated again and, after room temperature incubation, plates from two of the samples showed a very few ropy colonies. Both of the samples were drawn directly from the udder into sterile bottles, the udder having been previously washed with a disinfecting solution in the case of one sample, but not in the case of the other. Samples taken from the milking machine which was regularly used on the farm and in various other ways failed to show ropy organisms with the methods of examination used. The data obtained indicate that the organisms may have been gaining entrance to the milk in the udder; the results, however, are not at all conclusive because the examination was made at a time when ropiness was not developing in the milk held under practical conditions and it is entirely possible that the organisms may have gained entrance to the udder subsequent to their appearance in the general milk supply. It is rather surprising that the ropy organisms were not isolated from all of the samples instead of only those drawn from the udder in such a way as to largely or entirely eliminate outside contamination; this may, however, have been due to an overgrowth with other organisms in the case of the samples not drawn so as to limit contamination from external sources.

The first report of the ropy condition under consideration was received early in January, 1920. The time of year suggests an unusual source of contamination, since most of the outbreaks of ropy milk occur during the warmer months when infection from surface water, etc., is more likely to occur, and favors the idea of the infection of the milk from the interior of the udder.

If the udder was the true source of the organisms causing the abnormality, it must have been eliminating them in larger numbers than it was at the time the samples secured in various ways were examined or, what is very improbable, the milk must have been held under conditions more favorable for the growth of the ropy organism than those under which the samples were held.

The ropy organism isolated produced a ropy condition in whole milk whether unheated, pasteurized, or sterilized, in a considerable number of trials at both 20° and 37°C. In the unheated and pasteurized whole milk the ropiness was largely confined to the cream layer and even when the cream layer was very ropy the milk below was of a normal consistency; at both temperatures after the first appearance of ropiness the condition increased for a time and then decreased, the decrease apparently being hastened by the development of acid. With sterile whole milk the ropiness was more pronounced in the cream layer, which was very narrow as a result of the heating, but the milk below also showed this condition. The ropiness seemed to increase and then decrease in the sterile milk in much the same way as in the unsterile. With all the different lots of whole milk the ropiness was greater at 20°C. than at 37°C.

In sterile skimmed milk, ropiness developed rapidly in the upper layers and was already evident in test tubes in six to eight hours at 20°C. At 37°C. ropiness also developed rapidly but was never as pronounced as at 20°C. There seemed to be an increase and then a decrease in the ropiness although in tubes at 20°C. the material was still ropy when digestion was practically complete.

As has already been stated, the ropy organism rapidly digested sterile skimmed milk in tubes held at room temperature. At 37°C., however, inoculated sterile skimmed milk did not undergo a rapid digestion, but soon showed a definite coagulation which acid determinations proved was not due to the acidity developed; on standing there was some increase in acidity but even after considerable periods of time the acidity was not high enough to cause coagulation. For example, in a small bottle of inoculated sterile skimmed milk the acidity after twenty-four hours, which

was about the time of coagulation, was 0.31 per cent, calculated as lactic acid, while after nineteen days it was 0.47 per cent.

There are apparently factors other than temperature which influence the rate of digestion. Tubes of sterile whole milk which, as a result of the fat layer, presumably had a smaller air supply than did tubes of skimmed milk showed a much slower digestion than did the latter. Containers having a deeper layer of skimmed milk than tubes also seemed to show a slower digestion.

Since the ropiness occurred at a time when it would be expected that the milk was being held at a fairly low temperature, the ability of the organism to produce ropiness in milk at approximately 10°C. was tried. A ropy condition developed in inoculated sterile milk held at this temperature but comparative tests with a culture of *Bact. viscosum* showed that this organism produced ropiness in milk in a shorter time at approximately 10°C. than did the coccus form. Both organisms, however, eventually produced a very ropy condition.

The organisms usually responsible for ropiness in milk are definite capsule formers, at least in young cultures. With the coccus form isolated, capsules were not observed, but it seemed that a product that was viscous was being secreted by the organisms, since strands of well-stained material were commonly found stringing out from the cells and often running from one cell to another. The microscopic pictures obtained suggested that material quite like the capsular material of *Bact. viscosum* was secreted but was rapidly taken up by the surrounding medium instead of being retained near the cell in a definite capsule. The situation with the coccus form seems to be much like that with such typical capsule formers as *Bact. viscosum* at the time of very pronounced ropiness, when the capsular material is strung out through the medium instead of being retained in a definite form.

The organism responsible for the outbreak of ropy milk was studied morphologically, culturally, and bio-chemically and the data secured were compared with the published descriptions of the organisms reported as causing ropiness in milk. The comparison indicates that the organism isolated is closely related

to *M. mucofaciens* of Thöni and Thaysen<sup>2</sup> which was isolated from ropy milk in Berne and is also somewhat like *M. Freudenreichii* of Guillebeau,<sup>3</sup> an organism that is commonly the cause of ropy milk in Switzerland. Perhaps the most striking difference between the isolated type and *M. mucofaciens* is the color-producing power of the latter; while this may not be an important difference, the work of Winslow, Rothberg and Parsons<sup>4</sup> indicates that color production is definite and constant among the white and orange cocci, and accordingly the organism isolated is considered to be an undescribed species and the name *Staph. cremoris-viscosi* is proposed for it.

#### DESCRIPTION OF STAPH. CREMORIS-VISCOSE

##### *Morphology*

*Form.* The organism was definitely spherical.

*Size.* The organism showed but little variation in size on different media and at various ages. The average cell had a diameter of about 0.9 to 1 micron and the extremes varied from about 0.7 to 1.2 microns.

*Arrangement.* The organisms were irregularly arranged with isolated cells very common in all the usual media.

*Motility.* Motility was not observed in young bouillon cultures held at either 20° or 37°C.

*Staining reaction.* The organism stained readily with the ordinary stain and was definitely Gram + with only an occasional Gram - cell even in old cultures.

*Spore formation.* Spores were not observed and the organisms taken from cultures on various media and at different ages failed to resist 80°C. for ten minutes.

##### *Cultural characteristics*

*Beef infusion agar slope.* After twenty-four hours at room temperature or 37°C. there was a very heavy, white, viscous growth, slightly raised and with a smooth edge. There was but little change with an increase in age.

<sup>2</sup> Centbl. f. Bakt., Abt. 2, xxxvi, 359.

<sup>3</sup> See Orla-Jensen, Die Bakt. in der Milchwirtschaft, 1913, 79.

<sup>4</sup> Jour. Bact., 1920, v, 145.

*Beef extract agar slope.* Growth on beef extract agar was of essentially the same character as that on beef infusion agar but was slower.

*Whey agar slope.* On whey agar, growth was much slower than on beef infusion agar but eventually was of the same general type.

*Beef infusion agar stab.* Beef infusion agar stabs gave a growth along the line of puncture that at first had the appearance of closely aggregated colonies and later showed tiny projections with rounded ends. There was a heavy, white, viscous surface growth that was raised and had a smooth edge. The type of growth was the same at both room temperature and 37°C.

*Beef extract agar stab.* Growth was of the same general type as that in infusion agar stabs.

*Whey agar stab.* Growth was essentially the same as that in infusion agar stabs, but considerably slower.

*Beef infusion agar plate colonies.* On beef infusion agar plates colonies were evident after twenty-four hours at room temperature and after forty-eight hours were well differentiated into surface and sub-surface colonies. The surface colonies were large, round, viscous, white, very opaque, raised and smooth-edged while the sub-surface colonies were smaller, round to ellipsoidal, viscous, white, compact and smooth-edged; with an increased incubation there was an increase in the size of the colonies until those at the surface were from 2 to 3 mm. in diameter and those beneath the surface were up to 1 mm. in their longest dimension. At 37°C. the development of colonies on beef infusion agar was essentially the same as at room temperature.

*Whey gelatine stab.* Growth developed along the stab and then liquefaction began after several days at 20°C. Liquefaction proceeded only slowly and was greater at the surface than down in the medium; the liquefied portion of the gelatine showed a sediment. The entire medium was usually not liquefied even after two months' incubation.

*Bouillons.* Plain bouillon and bouillons containing various fermentable materials showed a turbidity and slight sediment in twenty-four to forty-eight hours at room temperature or 37°C. With age the turbidity cleared and the sediment increased. The sediment seemed to be viscous and was drawn out into threads on agitation.

*Potato.* A white, moist, somewhat viscous, spreading growth occurred on potato at both room temperature and 37°C. but development was slow.



*Dunham's solution.* After 24 hours at room temperature or 37°C. there was a turbidity and slight sediment. With an increase in age there was a greater turbidity and an increased sediment and later the turbidity cleared up leaving only a sediment. At all stages, the sediment was viscous and when brought to the surface could be drawn out into threads.

*Uschinsky's solution.* There was no evidence of growth at either room temperature or 37°C. even after long periods of incubation.

*Plain milk.* At room temperature plain skimmed milk quickly became ropy and then digested, the digestion in tubes being almost complete in three or four days. When fat was present digestion was much slower. At 37°C. plain milk became ropy and then soon coagulated with apparently some digestion or at least an expression of whey.

*Litmus milk.* The changes were essentially the same as in plain milk except that the litmus was reddened in the milk digested at room temperature while it was reduced in the milk coagulated at 37°C.

*Peptone (1 per cent) milk.* Growth was essentially the same as in milk without peptone.

*Peptone (1 per cent) litmus milk.* Growth was essentially the same as in litmus milk without peptone.

### *Bio-chemical features*

*Gas production.* No evidence of gas production was observed in milk or in bouillons containing glycerol, fructose, galactose, glucose, lactose, maltose, sucrose, mannitol, salicin, raffinose, inulin or starch.

*Acid production.* In milk there was a slight increase in acidity, the maximum acidity observed being 0.47 per cent, calculated as lactic acid. In bouillons containing the various fermentable materials listed above there was very little if any acid development, 0.5 per cent N/1 acid being the maximum found.

*Indol production.* Indol was not detected after various incubation periods at room temperature and 37°C.

*Oxygen relation.* The organism was definitely aerobic.

### SUMMARY

An outbreak of ropy milk occurring in Iowa, that was unusual in the time of year it appeared, was studied and found to be due to an organism quite different than the type usually responsible

for ropy milk. The organism, which was studied morphologically, culturally, and bio-chemically, was closely related to *M. mucofaciens* (Thöni and Thaysen) and was also somewhat like *M. Freudenreichii* (Guillebeau) but showed characters that made it seem desirable to designate it as a new species, *Staph. cremoris-viscosi*. Some time after the disappearance of the trouble the causative organism was found in the udders of cows in the producing herd. It was impossible, however, to tell whether the invasion of the udders preceded or followed the outbreak of ropy milk; in either case conditions were different when the milk carefully drawn from the udders was examined than when the outbreak occurred since when the examination was made the milk was no longer developing ropiness.

# A COMPARATIVE STUDY OF CORN SILAGE IN CONCRETE AND STAVE SILOS<sup>1</sup>

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## INTRODUCTION

Owing to the continual increase in cost of feed for dairy cattle, silos are becoming more and more popular. While the wooden or wooden-stave silo is the prevailing type in most sections of the country, concrete silos have come into favor. There seems, however, to be a rather general feeling that the silage produced in concrete silos is inferior in quality to that produced in the stave silo. The purpose of the investigation described in this paper is an attempt to throw some light on this phase of the silo problem.

## PREVIOUS INVESTIGATIONS

In 1884, Adolph Mayer, at Wageningen (1), reported the analysis of green maize put in, and maize silage taken out of four silos. In two unlined earth-pit silos containing, at ensiling, respectively 6 tons and 12 tons of maize which remained in the pits four and one-half months and five months, he found a loss of 40 per cent and 37 per cent respectively in food value. By ensiling 12 tons of maize for three months in a double-walled ice house he obtained an 18 per cent loss of food value. Using a cement silo with a roof he found a loss, after six and one-half months, of 36 per cent of the food value of the 6½ tons of maize ensiled.

In 1914 at the Iowa Agricultural Experiment Station, Neidig (2) used three types of round silos, brick, wood-stave, and concrete, having a capacity of 83 tons, 131 tons, and 138 tons, respectively. He decided, after careful study of the results from

<sup>1</sup> Published by permission of the Secretary of Agriculture.

the three silos, that the type of silo has no appreciable effect upon the keeping quality of the silage. He stated among his conclusions:

The results show that no differences were noted in the chemical changes in the three silos which could be attributed to the effect of the different types of building materials upon the process of silage formation. It is readily seen that approximately the same results are obtained in the temperature observations, gas analysis, and volatile and non-volatile acids of silage from the three silos. The only differences noted are differences in the quantity; the ratio of chemical substances is very nearly the same for each silo.

He stated further in discussing the relative merits of the different types of silos

The chief factors necessary for the production of good silage are, therefore; smooth, air-tight walls, corn in the right state of maturity, the proper amount of moisture, and carefulness in filling.

During 1913 and 1915, Eckles, Oshel, and Magruder (3) studied temperature changes and chemical changes in a number of types of farm silos, experimental silos, and experimental cans, including two concrete silos and three stave silos. The concrete silos had a capacity of 168 tons each and the stave silos had capacities of 190 tons for one and about 150 tons each for the other two.

The authors concluded:

The temperature in the silage in the early stage is influenced to some extent by the temperature of the atmosphere at the time of filling and of the water used, if any.

The material used in construction of the silo has but little, if any, influence upon the temperature of the silage.

Analyses of silage from the wall and center of silos of various types of construction showed no difference in composition due to the material used in the construction of the silo.

## PRESENT INVESTIGATION

*Silos used*

The silos used in the investigation were located at the Dairy Division Experimental Farm, Beltsville, Maryland. They held approximately 150 tons of silage each. They were built side by side and consequently were exposed to practically identical weather conditions with the exception that the stave silo was situated directly south of the concrete one and consequently was somewhat more sheltered from northerly winds. The stave silo was also exposed to more heat of the sun.

*Temperatures of silos*

Temperatures were taken by means of electrical thermometers buried in the silage, with cables leading to temperature indicators where the readings were taken at regular intervals. Three of these thermometers were used in the stave silo and three in the concrete. They were buried at about the same distance from the top in each silo, so that the pressure of silage would be approximately the same at the point at which each of the thermometers was buried. One thermometer was 3 inches from the wall, another 18 inches, and the third in the center of each of the silos. A mercury thermometer was used in taking the temperature of the atmosphere at the time the readings were taken.

*Changes in chemical composition*

In order to determine the factor of chemical composition a sample of cut corn was analyzed at the time of filling and a sample of the silage after fermentation had ceased. The plan was to run into the silo a considerable quantity of cut corn, thoroughly mix it on a piece of canvas, take a sample for analysis, and fill a number of sacks with a known weight of the cut corn for burial at certain distances from the silo wall. These sacks were made of cheesecloth and a sack was buried at the side of each thermometer so that the centers of the sacks were the same distance from the wall as were the thermometers. In order to get these

sacks buried at the proper places a metal form 6 by 12 by 18 inches and open at both ends was used, into which the sacks were tightly pressed. Cut corn of the same lot from which the sacks were filled was used to fill in around the forms. The forms were then removed. As the sacks were at the same level as the thermometers and the same distance from the wall the temperature as shown by the thermometers could be used in determining the effect of different temperatures upon the chemical composition of the silage contained in the sacks. These samples were removed for analysis as soon as the silage was fed down to the place where they were located. Analyses of both the original and fermented materials were made for kind and amount of acids, water, ash, crude fiber, total nitrogen, albuminoid nitrogen, ether extract, and nitrogen free extract.

### *Quality of the silage*

The quality of the silage was judged by the appearance, odor, and palatability when fed to cows. It was originally planned to conduct an actual feeding trial to determine the relative nutritive value of the two classes of silage in case the chemical composition and the quality of the silage indicated the advisability of so doing. As no marked difference was observed in the quality of the silage made in the two silos it was decided not to conduct such a feeding experiment.

### *Methods of analysis*

The material on reaching the laboratory was first thoroughly mixed. One kilogram of the mixed material was dried in a steam drying closet at from 50° to 60°C. to constant weight. It was then exposed to the air in the laboratory for a few days, the final weight being taken to represent the air-dry condition. The air-dry material was ground in a power grinder to a fine powder suitable for analysis. Another part of the fresh material was pulped in a power meat grinder, thoroughly mixed, and samples taken immediately for the nitrogen and volatile-acids determinations.

The volatile acids were determined by the method of Duclaux. All other determinations were made according to the methods of the Association of Official Agricultural Chemists.

Two years were covered by the investigation. As the silos were being filled the first season, a breakdown in the cutting machinery after the sacks were placed caused an interruption. The corn which was put into the upper parts of the silos was consequently considerably more mature than that put into the lower parts and it was considered necessary to water both silos. Since both received the same treatment it is not thought that this interfered with the comparative results.

The sacks were placed in both silos, the first season, on October 7. They were removed between March 7 and March 14, having been in the silos 151 to 158 days. The sacks were placed in the silos the second season on September 26. Owing to feeding conditions at the farm the level of the sacks was reached much earlier than in the first season. They were removed between October 22 and October 29, having been in the silos 26 to 33 days.

### *Results of analyses*

The following tables give the chemical results on the material as it was ensiled and as it was removed, and also give the temperatures.

*The temperatures.* For the greater part of the first season temperature readings were taken each day at 6 p.m. Toward the last of the season they were taken less frequently. During the second season, which extended but a little more than a month, the readings were taken at 5 p.m. each day, with one or two exceptions. Unabridged tables containing these data would take up too much space to be presented here. The tables which follow contain figures representing average daily temperatures for each week of the active ensiling period and it is believed that they will serve as well as the much more cumbersome ones giving the daily temperatures.

In comparing the temperatures of the two silos it should be borne in mind that the stave silo was situated directly south of

the concrete silo and received more or less protection from northerly winds and more heat from the sun. This will account, perhaps, for the somewhat lower temperatures in the concrete silo.

## SUMMARY AND CONCLUSIONS

Two 150-ton silos, one wood-stave and one concrete, were used in the investigation. They stood side by side and were

TABLE 1

*Chemical composition of material when placed in both silos and when removed; first season*

CONSTITUENT	GREEN MATERIAL WHEN PUT INTO SILO	SAMPLE NO. 1 STAVE SILO, NEXT TO WALL	SAMPLE NO. 2 STAVE SILO, 18 INCHES FROM WALL	SAMPLE NO. 3 STAVE SILO, CENTER	SAMPLE NO. 4 CON- CRETE SILO, NEXT TO WALL	SAMPLE NO. 5 CON- CRETE SILO, 18 INCHES FROM WALL	SAMPLE NO. 6 CON- CRETE SILO, CEN- TER
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Moisture.....	81.71	80.45	81.95	80.43	82.02	83.23	81.69
Ether extract .....	0.75	0.68	0.80	0.78	0.74	0.64	0.59
Total nitrogen.....	0.317	0.261	0.262	0.269	0.259	0.256	0.290
Albumin nitrogen.....	0.256	0.204	0.170	0.178	0.171	0.149	0.168
Crude fiber.....	6.07	5.30	4.94	4.57	4.37	4.54	4.41
Furfural.....	2.09	2.27	1.93	2.15	1.91	1.81	1.94
Starch.....	3.08	3.18	3.25	3.63	3.12	2.95	3.08
Total sugars as dextrose.....	0.37	0.025	None	None	0.055	None	None
Invert sugars as dextrose.....	0.04	None	None	None		None	None
Ash.....	1.36	1.26	1.31	1.43	1.32	1.18	1.17
		cc.	cc.	cc.	cc.	cc.	cc.
Volatile acids in 100 grams*.....		72.36	124.5	115.0	99.97	121.9	149.0

\* These figures refer to the quantity of  $\frac{N}{10}$  Ba(OH)<sub>2</sub> solution required to neutralize the total volatile acids in 100 grams of pulp.

exposed to the same temperature and weather conditions, with the exception that the stave silo, which was located to the south of the concrete silo, received some protection from northerly winds and more heat from the sun.

When the silos were about half filled, sacks of carefully mixed cut corn were buried near the wall, 18 inches from the wall, and in the center of each silo. Temperatures were taken of the



TABLE 2

*Chemical composition of material when placed in both silos and when removed; second season*

CONSTITUENT	GREEN MATERIAL WHEN PUT INTO SILO	SAMPLE NO. 1 STAVE SILO, NEXT TO WALL	SAMPLE NO. 2 STAVE SILO, 18 INCHES FROM WALL	SAMPLE NO. 3 STAVE SILO, CENTER	SAMPLE NO. 4 CON- CRETE SILO, NEXT TO WALL	SAMPLE NO. 5 CON- CRETE SILO, 18 INCHES FROM WALL	SAMPLE NO. 6 CON- CRETE SILO, CEN- TER
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Moisture.....	78.09	79.18	78.00	78.34	78.50	78.60	77.48
Ether extract.....	0.29	0.47	0.47	0.43	0.53	0.46	0.50
Total nitrogen.....	0.445	0.427	0.408	0.421	0.435	0.435	0.421
Albumin nitrogen.....	0.424	0.381	0.352	0.368	0.381	0.356	0.370
Crude fiber.....	4.97	4.23	4.40	4.24	4.28	4.41	4.83
Furfural.....	2.49	2.15	2.25	2.20	2.30	2.22	2.12
Total sugars as dextrose.....	1.65	0.16	0.26	0.69	0.18	0.43	0.39
Invert sugars as dextrose.....	0.77	None	None	None	None	None	None
Ash.....	0.94	1.02	0.94	0.90	1.01	0.97	0.96
Volatiles acids in 100 grams ground pulp*.....		cc.	cc.	cc.	cc.	cc.	cc.
		67.1	56.24	56.3	64.7	64.5	61.0

\* These figures refer to the quantity of  $\frac{N}{10}$  Ba(OH)<sub>2</sub> solution required to neutralize the total volatile acids in 100 grams of pulp.

TABLE 3

*Volatile acids in silage from the two silos*

ACID	SAMPLE NO. 1 STAVE SILO, NEXT TO WALL	SAMPLE NO. 2 STAVE SILO, 18 INCHES FROM WALL	SAMPLE NO. 3 STAVE SILO, CENTER	SAMPLE NO. 4 CON- CRETE SILO, NEXT TO WALL	SAMPLE NO. 5 CON- CRETE SILO, 18 INCHES FROM WALL	SAMPLE NO. 6 CON- CRETE SILO, CEN- TER
First season						
	per cent	per cent	per cent	per cent	per cent	per cent
Acetic acid.....	1.84	3.49	2.72	2.47	3.73	2.92
Propionic acid.....	0.24	0.41	0.38	0.50	0.37	0.24
Second season						
Acetic acid.....	1.54	1.26	1.19	1.39	1.49	1.35
Propionic acid.....	0.38	0.16	0.19	0.22	0.08	0.05
Formic acid.....						0.04

outside air and of the silage at the points where the sacks were buried in each silo. The silage in the sacks was subjected to a rather complete chemical analysis, including the volatile acids. Neither the temperatures nor the chemical analysis revealed any marked difference in the quality of the silage that could be ascribed to the material used in the construction of the silo. Cows ate the silage from both silos with the same avidity.

TABLE 4

*Mean temperatures of the air and of the silage at three different points in each silo*

WEEK ENDED	AIR	SAMPLE NO. 1 STAVE SILO, NEXT TO WALL	SAMPLE NO. 2 STAVE SILO, 18 INCHES FROM WALL	SAMPLE NO. 3 STAVE SILO, CENTER	SAMPLE NO. 4 CON- CRETE SILO, NEXT TO WALL	SAMPLE NO. 5 CON- CRETE SILO, 18 INCHES FROM WALL	SAMPLE NO. 6 CON- CRETE SILO, CEN- TER
First season							
	deg. C.	deg. C.	deg. C.	deg. C.	deg. C.	deg. C.	deg. C.
October 13.....	21.3	25.3	31.8	33.0	21.2	26.8	28.6
October 20.....	16.6	23.0	34.1	37.3	18.6	28.3	32.5
October 27.....	14.5	20.2	30.6	36.2	16.4	26.3	34.8
November 3.....	16.0	18.0	27.6	35.3	14.1	24.5	35.6
November 10.....	14.6	15.4	25.3	34.7	12.3	22.5	34.7
November 17.....	13.5	14.8	23.6	34.1	12.1	20.9	33.8
November 23.....	13.9	12.2	22.2	33.4	8.9	19.4	32.5
December 1.....	5.5	9.4	20.3	32.5	6.1	17.3	30.8
December 7.....	8.7	10.8	18.3	31.3	8.7	15.3	29.0
December 14.....	2.7	7.5	17.7	30.7	4.5	14.5	27.7
December 21.....	7.1	7.1	16.0	29.4	4.0	12.6	26.3
Second season							
October 3.....	21.6	28.7	34.5	35.2	23.1	29.1	29.2
October 10.....	22.3	24.5	32.6	37.0	19.9	27.1	29.1
October 17.....	18.7	21.7	30.3	37.0	16.9	24.7	28.5
October 24.....	14.0	20.5	28.9	37.3	13.8	22.6	28.3

## REFERENCES

- (1) MAYER, ADOLPH: Beitrage zu einer genaueren Kenntniss der Vor und Nachtheile des Einsauerung Processen. Jour. für Landwirthschaft, 1884, xxxii, 357-405.
- (2) NEIDIG, RAY E.: Chemical changes during silage formation. Research Bulletin No. 16, Iowa Agricultural Experiment Station, July, 1914.
- (3) ECKLES, OSHEL, AND MAGRUDER: Silage investigations. Research Bulletin No. 22, Missouri Agricultural Experiment Station, March, 1916.

## HOW DO BACTERIA GET INTO MILK AT THE FARM AND HOW MAY THEIR NUMBER BE REDUCED?

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The high germ content of city milk supplies has been the occasion of much alarm for the past thirty years. Many cities (1) have attempted to meet the situation by declaring that milk containing more than some definite maximum of bacterial life should be excluded from the retail market.

About twenty years ago there began in this country a series of fruitful attempts (2) to find the actual sources of bacterial life in milk. While there has been a general and growing interest in this line of studies the work has been most intensively pushed at the New York Agricultural Experiment Station, at the Federal Dairy Division, and at the University of Illinois.

But little study was needed to show that the bacteria present in the milk as it reached the city plant were due to two distinct sources; first, the seeding with germ life which the milk receives at various stages in its journey, and second, the growth which takes place after the germs are seeded into the milk.

It has long been known that for some time after the milk is drawn from the cow the germ content ordinarily decreases (3), the extent and duration of this decrease depending upon a number of factors. Gradually the multiplication of some of the germs which have fallen into the milk compensates for the decrease of the others. As a result of the action of various factors, the germ content of the morning's milk as it is delivered from the farm to the bottling plant or the shipping station ordinarily represents little more than the number of bacteria seeded into the milk.

Milk companies in increasing numbers are determining the germ content of the milk as it comes to them. While their

findings are subject to fairly wide variations at any given plant and there are also variations in different parts of the country, 50,000 bacteria per cubic centimeter may be taken as a very conservative figure for the bacterial content of morning's milk as delivered at the shipping station or bottling plant during warm weather.

The problem of the dairy bacteriologist is first to find the sources of this large number of bacteria which get into milk and second, to find practical means by which the introduction of germ life can be markedly reduced. At the beginning of the studies concerning the sources of the bacteria added to milk, there was a general feeling that the character and condition of the barn was mainly the responsible factor. This feeling found expression quite frequently in summary orders from health departments to milk producers either to provide better barns or quit furnishing milk for the municipal supply.

Investigation soon made it evident that the construction of the barn (4), within any reasonable limits, exerted little or no influence upon the germ content of the milk. It was found further that while many of the ordinary barn practices exerted a measurable influence upon the amount of germ life in the milk (5) the magnitude of those influences was plainly secondary. Perhaps their most constructive contribution consisted in pointing out that at least 80 per cent of the germ life getting into milk comes ordinarily from the utensils (6) in which the milk is handled and that among the utensils used at the farm the milk can is ordinarily the major source of germ life.

Having shown that the milk cans, and not the barns, are mainly responsible for the large amount of germ life added to the milk at the farm, the next problem was to find practical means of reducing the germ life in the milk cans. Data were presented which indicated that the germ content of cans washed at the milk plant is progressively reduced when the cans are thoroughly washed, when they are rinsed with clean water, and particularly when they are thoroughly steamed. The data further showed that the amount of germ life in the cans when used later at the farm is largely dependent upon the presence

and area of moist surface persisting in the interior of the cans. These results suggest that if the cans which are now washed and steamed at the milk plants are properly dried before being returned to the milk producers, the germ content of the milk later returned to the milk plants in these same cans will be markedly reduced.

While the solution of the problem of producing milk of low germ content is thus shown to lie largely within the control of the milk plant operator, the fact remains that under present conditions a large part of the cans returned to the producer from the milk plant are returned in a moist condition. Accordingly it is important to know what the milk producer can do with these moist, high germ content cans in order to best prepare them for receiving milk. Moreover, the problem of the most effective, and at the same time the most practical method of handling pails and similar utensils is one constantly present on all farms.

The mechanical washing of pails and cans on dairy farms is both so simple and ordinarily so well done as to need little comment. Moreover, following this washing there is much divergence of practice with regard to subsequent rinsings of the washed utensils with hot water. A large part of the effect of such rinsing upon the germ life has been found to result from the mechanical<sup>1</sup> removal of germ life by the rinse water. The magnitude of this mechanical removal has been measured by experiments in which rinse water at 70°F. was used. The results of such treatment of a considerable number of well-washed 8-gallon cans suggests that more than two billion germs are thereby removed from each can.

A somewhat larger number of germs is mechanically removed by using warmer rinse water, but, as the temperature of the rinse water increases, the measurement of the germs actually removed is complicated by the destructive action of the hot water upon the germs. For example, rinsing with water at 205° to 208°F. undoubtedly removes mechanically more germs than a cooler rinse water will remove, but the destructive action of this high temperature is so great that the number of living germs found in the rinse water rarely exceeds a half billion per can.

<sup>1</sup> This and the following statements regarding results of investigations refer to data given in Ill. Agr. Exp. Sta. Bul., now in press, on Elimination of germs from dairy utensils. I. By rinsing. II. By drying in sun and air.

The destructive effect of hot rinse water upon germ life is much less than is commonly supposed, being much reduced by the rapid cooling of the rinse water through the transfer of its heat to the utensil. Thus when an eight-gallon can at 72°F. is rinsed with a quart of water at 150°F., the temperature of the rinse water falls about 40° within sixty seconds. As the amount of rinse water per can is increased, the drop in temperature is correspondingly reduced, so that when 4 quarts of water at 150°F. are added to a can the temperature drops only about 20°.

When boiling water is applied the destruction of germ life is pronounced, particularly when more than two quarts of rinse water are used per can. Under such circumstances the combined effect of the mechanical removal and the destruction of germ life is such that if the cans were immediately filled with sterile milk the germs remaining in such scalded cans would commonly increase the germ content of the milk only about 100 bacteria per cubic centimeter. Accordingly the fairly common practice of applying scalding water to freshly washed utensils or to moist washed cans returned from the milk plant is to be commended. It should be noted, however, that the pouring of this rinse water from one can to another greatly reduces the effectiveness of this practice, both because the rinse water promptly becomes too cool to exert much killing effect and because it quickly becomes contaminated by the germ life which it mechanically removes from the utensils.

As a method of treating the moist, washed cans which arrive at the farm shortly before they are needed nothing better is available ordinarily than the thorough scalding of the can with hot water. However, when any considerable interval is to elapse before the cans are filled with milk, it is of prime importance that they should be promptly and thoroughly dried.

Observations have been made upon the effect of inverting washed and rinsed cans on a rack in the sun. When the sun is hot and the air dry the cans dry quickly and the germ life in the cans is promptly reduced. Cans which are fairly well rinsed, when thus dried and kept dry, are ordinarily in a condition to

add not more than 100 bacteria per cubic centimeter to the milk with which they are filled. However, during damp or rainy weather, when moisture remains in the cans the germ content holds its own or even multiplies considerably.

A further series of observations was made by adding to sterile cans 10 cc. from a vat of rinse water or wash water and holding the cans twenty-four hours, one half of the cans with the covers on and the other half with their covers removed. Where the covers remained on the cans, the germ life increased between 20 and 3000 fold. Where the covers were removed and the conditions permitted rapid drying, the germ content fell promptly, but where the drying was retarded the amount of germ life remained constant or even increased.

These studies of the source of the bacteria which get into the milk at the farm may be summarized by stating that evidently the milk cans are ordinarily the main factor in seeding the milk, and that during hot weather they normally contribute 30,000 or more bacteria per cubic centimeter to the fresh milk. This high germ content of the milk cans is ordinarily due, not to failure to properly wash and rinse the cans at the milk plant, though improvements at this point are undoubtedly needed in some cases, but primarily to the growth which takes place in the cans after the washing process.

The amount of growth occurring in the milk cans between the time they are washed and the time they are filled with milk depends upon the temperature, the amount of moist area within the cans and the length of time which elapses before the cans are used. Of these factors the moisture of the can is the only one which can be successfully controlled.

A thorough drying of the cans before they leave the milk plant will exert a profound influence upon the germ content of the milk later returned to the plant in these cans.

Where moist cans are returned to the producer he may put them into acceptable condition by thoroughly rinsing them with scalding water. If they are not to be used immediately, they should be promptly and thoroughly dried.

## REFERENCES

- (1) Bacterial Standards for Milk. U. S. Public Health Reports 29: 1218-1221. 1914.
- (2) RUSSELL, H. L.: The sources of bacterial infection and the relation of the same to the keeping quality of milk. Wis. Agr. Exp. Sta. Ann. Report 11 (1894): 150-165. 1895.  
BURR, R. H.: Investigations on the sources of the acid organisms concerned in the souring of milk. Storrs Agr. Exp. Sta. Ann. Report 13 (1900): 66-81. 1901.  
STOCKING, W. A., JR.: Efficiency of a covered pail in excluding filth and bacteria from milk. Storrs Agr. Exp. Sta. Ann. Report 14 (1901): 105-121. 1902.  
CONN, H. W., AND STOCKING, W. A., JR.: Comparison of bacteria in strained and unstrained samples of milk. Storrs Agr. Exp. Sta. Ann. Report 15 (1902-3): 33-37. 1903.
- (3) FOKKER, A. P.: Ueber bakterienvernichtende Eigenschaften der Milch. Zeit. f. Hygiene, ix, 41-55. 1890.
- (4) RUEHLE, G. L. A., AND KULP, W. L.: Germ content of stable air and its effect upon the germ content of milk. N. Y. Agr. Exp. Sta. Bul. 409. 1915.  
PRUCHA, M. J., AND WEETER, H. M.: Germ content of milk. I. As influenced by the factors at the barn. Ill. Agr. Exp. Sta. Bul. 199. 1917.
- (5) STOCKING, W. A., JR.: Quality of milk affected by common dairy practices. Storrs Agr. Exp. Sta. Ann. Report. 18 (1906): 66-90. 1907.  
HARDING, H. A., RUEHLE, G. L., WILSON, J. K., AND SMITH, G. A.: The effect of certain dairy operations upon the germ content of milk. N. Y. Agr. Exp. Sta. Bul. 365. 1913.  
PRUCHA, M. J., AND WEETER, H. M.: Germ content of milk. I. As influenced by the factors at the barn. Ill. Agr. Exp. Sta. Bul. 199. 1917.
- (6) PRUCHA, M. J., HARDING, H. A., AND WEETER, H. M.: Utensils as a source of bacterial contamination of milk. Sci. N. S. xlii: 353. 1915.  
PRUCHA, M. J., WEETER, H. M., AND CHAMBERS, W. H.: Germ content of milk. II. As influenced by the utensils. Ill. Agr. Exp. Sta. Bul. 204. 1918.  
AYERS, S. H., COOK, L. B., AND CLEMMER, P. W.: The four essential factors in the production of milk of low bacterial content. U. S. Dept. Agr. Bul. 642. 1918.





# THE EFFICIENCY OF MILK SUBSTITUTES IN CALF FEEDING<sup>1</sup>

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## INTRODUCTION

For many years there has been a desire among dairymen to secure a milk substitute for the rearing of young calves. This desire and need have increased as the cost of milk production has increased. During our recent war the demand for whole milk increased greatly and consequently the price of milk and its by-products advanced to such an extent as to make the expense of raising calves on whole milk almost prohibitive. Although skim milk has proved to be an excellent substitute for whole milk after the calf has reached the age of two to three weeks, the supply of skim milk also is limited especially by dairymen who supply the customer with whole milk. There is no doubt but that whole or skim milk is the ideal food for the growing animal. The only difference between whole milk and skim milk is the fat content, so that in feeding skim milk the nutritive value of the fat must be supplied by other feeds such as corn, oats, etc. The grains supply the carbohydrates which serve as a source of energy. A rational feed to insure growth, maintenance and health must contain an ample quantity and quality of food materials, also a sufficient amount of both types of vitamins, the "fat soluble A" and "water soluble B," in addition the requisite amount of mineral matter.

## RECENT INVESTIGATION

For many years nutritional investigators were of the opinion that the bodily growth and well-being of animals were dependent primarily on properly balanced rations and that such rations

<sup>1</sup> Acknowledgment is given Mr. R. E. Caldwell for his help during the first and second part of this experiment.

would meet the requirements and be considered complete foods if they consisted of certain definite proportions of any fats, proteins, carbohydrates and mineral salts. While in a large number of feeding experiments the growth obtained went far to prove the theory advanced, more recent investigations have shown conclusively that the above hypothesis is not valid, especially in regard to the proteins. Proteins are complex organic compounds built up of a large number of simpler definite chemical bodies called amino acids which have been designated by the term "Bau Steine" or building stones. It has been found that proteins derived from different sources vary considerably in quantity and kind of amino acids they yield. In some feeds certain amino acids are wanting, or if present are not in sufficient quantity. It is the kind and quantity of amino acids which give quality to the different proteins. Two amino acids play an especially important rôle in the growth and maintenance of animals. These are lysin and tryptophane. Therefore there are important differences in feeds resulting from the character of proteins derived from different sources. Numerous experiments have shown that there are great differences in the amounts of different proteins required to maintain animals without loss of body weight. It was found at the Wisconsin Experiment Station that there was a great difference in the value of proteins when derived from different sources. Experiments showed that the pig was able to retain for the construction of body tissue the following percentages of proteins in the naturally occurring food stuffs.

	<i>per cent</i>
Oil meal protein.....	16-17
Wheat <sup>2</sup> protein.....	20
Corn <sup>2</sup> protein.....	24
Oat <sup>4</sup> protein.....	25
Wheat germ protein.....	40
Skim milk protein.....	63
And a mixture of:	
Corn protein 90 per cent plus oil meal protein.....	10-31
Corn protein 75 per cent plus oil meal protein.....	25-37
Corn protein 60 per cent plus oil meal protein.....	40-31

<sup>2</sup> Hart, E. B., and McCollum, E. V., *J. Biol. Chem.*, 1914, xix, 373.

<sup>3</sup> McCollum, Simmons and Pitz, *J. Biol. Chem.*, 1916-1917, xxviii, 153.

<sup>4</sup> McCollum, Simmons and Pitz, *J. Biol. Chem.*, 1917, xxix, 341.

This table shows that there is a great difference in proteins derived from different sources, and suggests the possibility of combining two or more groups of proteins which will be more efficient in promoting growth than the proteins in the individual grains. From these data and numerous other experiments the results of which are more or less relative, it is evident that to base the protein content irrespective of the character will lead to disappointing results in the feeding of animals.

VITAMINES OR FOOD ESSENTIAL "FAT SOLUBLE A" AND "WATER SOLUBLE B"

The most recent investigations have shown that in addition to a well balanced ration of fats, carbohydrates, proteins and salt of proper quality there must be present in a complete feed other nutritional essentials in order to promote growth, maintenance and well-being of animals. These substances are popularly designated by the term "vitamines." The term "vitamines" was suggested by Casimir Funk as one to designate a number of substances of unknown chemical composition to which he ascribed the property of preventing, curing or controlling diseases such as scurvy, pellagra, polyneuritis, etc. To a lack of vitamins in the diet these deficiency diseases were attributed. Researches by McCollum tend to establish the existence of two types of these food essentials. These he has designated as "fat soluble A" and "water soluble B." Vitamines occur in all plants and animals in varying amounts. The "water soluble B" is abundant in nearly all natural foods, but is almost entirely absent in purified sugar, starch, polished rice and fats of both vegetable and animal origin such as lard, tallow, olive oil, cottonseed oil, etc. The yolk of eggs, fats of internal organs, as the kidney and heart, are relatively rich in the "fat soluble A." Those foods containing relatively small amounts of the fat soluble accessory are cabbage, turnips, carrots and most other root crops, and all highly sterilized products.

The leafy portion of growing plants is relatively rich in vitamins, differing markedly from the seeds. Rations of seeds,

tubers, and roots, although supplemented with the required mineral salts, are insufficient to maintain life, but the addition of the leafy parts of plants to this combination will furnish a food which is adequate to support life.

Milk, the best of natural foods, is rich in all essentials for the maintenance, growth and well-being of animals. It includes large amounts of both the fat soluble and water soluble vitamins.

#### MINERAL REQUIREMENTS OF THE ANIMAL

The growth of an animal involves the utilization of mineral matter in the formation of tissue, as well as of the skeleton. The greatest need is for bone formation. About two-thirds of the dry matter of bone consists of mineral matter. So it will be seen readily that it is very important that there should be a sufficient quantity of the mineral elements for the growing animals. These mineral elements are derived from the feed; and inasmuch as some feeds, especially grains, are very deficient in either one or more of the essential salts, it is highly essential to supply this deficiency. The more restricted the ration as to variety of feeds, the greater the danger of an insufficient supply of the proper mineral elements. Those most frequently deficient in feeds are sodium, calcium, and phosphates.

#### CALF FEEDING EXPERIMENTS

In 1915-1916 O. F. Hunziker and R. E. Caldwell conducted experiments in skim milk and milk substitutes for calf feeding. (Bulletin No. 193, this Station). Thirty calves of pure-bred stock were divided into lots of ten calves each. One lot was fed *skim milk*, another was fed *home mixed meal* and a third lot was fed Blatchford's Calf Meal. The following year, 1916-1917, O. H. Anderson, in coöperation with the authors, conducted a more extensive experiment on the efficiency of certain milk substitutes in calf feeding. These results were published in the Journal of Biological Chemistry, vol. xxviii, 1917. The object of this investigation was; first, to determine the growth efficiency of certain milk substitutes in calf feeding; second, to determine

the nitrogen distribution; third, to determine the nitrogen utilization of the different feeds used.

The following combinations were used in this experiment:

*I. Vegetable meal.* Linseed meal, soy bean meal, cottonseed meal and wheat middlings—equal parts by weight of each feed.

*II. Home mixed meal.* Hominy feed, linseed meal, cottonseed meal, White Swan flour, dried blood, equal parts by weight of each feed.

*III. Vegetable dried blood.* Soy bean meal, linseed meal, cottonseed meal, wheat middlings, and dried blood, equal parts by weight of each feed.

*IV. Home mixed casein meal.* Hominy feed, linseed meal, White Swan flour and casein, equal parts by weight of each feed.

From the results obtained by these preliminary experiments, especially from the work done by O. H. Anderson and others, it seemed advisable to continue this feeding experiment. The last experiment showed clearly that there was a great difference in the structure of the proteins as shown by the nitrogen retained for body growth and the distribution of the excreted nitrogen in the feces and urine.

#### PURPOSE OF EXTENDED EXPERIMENTS

The object of these experiments was to find a milk substitute which would be more efficient for the rearing of calves than the feeds already tried for this purpose. In order to determine the efficiency of any calf feed its success must be compared with that of milk as a standard. The experiment involved the comparison of the calves fed substitutes with those fed milk as to their growth and health, utilization and digestion of feeds, and the distribution of nitrogen in the urine and feces.

#### EXPERIMENT I

The investigators wished to test particularly the efficiency of liquid beef blood as compared with milk and also that of purely vegetable proteins. Analyses were made of all feeds or mixtures of feeds and an accurate record kept as to the amount fed. Total

nitrogen was determined in urine and feces of the daily composite. Feeding crates made it possible to collect urine and feces separately and keep a quantitative daily record of the nitrogen excreted.

### *Method of procedure*

The calves used in this experiment were grade Holstein, weighing 85.8 to 113.9 pounds and from six to nineteen days old. They were selected with respect to their uniformity in vigor and general condition. During the experiment period the calves were fed in crates. They were given frequent exercise on the barn floor and weighed every ten days. At the beginning they were fed on skim milk for a period of six to ten days. Then the milk substitutes were used as a part of the feed and gradually increased in such quantities as seemed compatible with the maintenance of the natural functional operations. At the end of ten days the calves that received milk substitutes were no longer fed any milk.

*Feeding chart for experiment I. Feeding periods and feeds received by each calf*

	CALF 22	CALF 110	CALF 219	CALF 223
Period I	Liquid blood Hominy Linseed meal Red Dog flour	Skim milk	Skim milk	Clover infusion Corn meal Gluten meal Red Dog flour Buckwheat flour
	Alfalfa hay	Alfalfa hay	Alfalfa hay	Clover hay
	Dry mash Ground oats and corn	Dry mash Ground oats and corn	Dry mash Ground oats and corn	Dry mash Corn gluten Corn meal Buckwheat, ground Red Dog flour

*Discussion of experiment I*

In planning this feeding experiment it was the aim to select a simple combination of grains which are available and can be purchased at a reasonable price. In this experiment a comparison has been made between the efficiency of the proteins derived from liquid blood in combination with vegetable proteins, the liquid blood supplying the animal proteins, and that of feeds composed entirely of vegetable proteins. The liquid beef blood was used in place of dried blood which is used considerably as a source of protein.

In drying blood, the proteins become insoluble in water, and owing to the high temperature to which the blood is subjected in drying and sterilization, it seems probable that some of its nutritional value might be destroyed. Liquid blood supplies the amino acids which, to a great extent, are required to synthesize the proteins of the animal. Besides, in the liquid blood the proteins are in their natural condition, and contain the food accessories in their active state.

Our feeding experiment bears out the superiority of liquid blood to dried blood in effecting metabolism, the physiological process by which non-living matter is built up into living matter or living matter broken down into more stable compounds. This difference is strikingly shown in the behavior of calf 22. When the calf was fed on the liquid blood ration, the nitrogen eliminated in the urine was 45.81 per cent of the nitrogen intake, while with the calves fed on vegetable proteins plus clover infusion and also milk, the nitrogen eliminated in the urine was 27.33 per cent of the nitrogen intake, and in the milk fed calves the nitrogen in the urine was 31.2 per cent. From our previous feeding experiment, it would seem that dried blood in combination with vegetable proteins had no more effect in stimulating metabolism than the feed composed entirely of vegetable proteins.

While it is realized that liquid blood is not practical as such, yet this experiment furnishes evidence of its superiority as a feed over dried blood and it is possible to prepare blood in a



dry and at the same time more soluble form, which, to a great extent, will have the nutritive value of liquid blood. In the feed composed of vegetable proteins the basic feed was similar in character of the proteins to that fed in the liquid blood mixture. The liquid used in the feed for calf 223 was an infusion of clover hay which in some communities is being used to supplement milk. The dry mash and roughage were also of similar character in the two lots. The clover hay infusion was heated to boiling and mixed with the requisite amount of dry basic feed. This food was prepared daily. Inasmuch as the ration of the two lots of calves consisted of grains of low feeding value and was deficient in both the unidentified dietary factors, "fat soluble A" and "water soluble B," and since the leaves contain the dietary factors in much greater quantity than the seeds, the aim was to supply these by the use of clover hay infusion. This experiment with the clover infusion was conducted primarily for the purpose to test the efficiency of purely vegetable proteins.

In feeding the calves, sufficient quantities of the mixtures were fed to keep the animals in normal condition, the physical condition of the feces being used as criterion. Dry mash and roughage were supplied in sufficient quantity to satisfy the wants of the animals.

### *Summary of experiment I*

I. The following represents the average daily gain in pounds of the three representative feeds, liquid blood, clover infusion, and milk, during the first ninety days, or the critical period of the life of the calf.

	<i>pounds</i>
Calf 22: Liquid blood ration . . . . .	1.32
Calf 219: Milk ration . . . . .	1.40
Calf 110: Milk ration . . . . .	1.17
Calf 223: Clover infusion ration . . . . .	1.03

II. The following per cent of nitrogen fed was retained.

	<i>per cent</i>
Liquid blood ration . . . . .	24.4
Milk . . . . .	42.8
Clover infusion ration . . . . .	31.9

III. Of the nitrogen intake, the excreted nitrogen was distributed as follows:

Liquid blood ration:

	<i>per cen</i>
In urine.....	45.9
In feces.....	29.9

Milk ration:

In urine.....	31.2
In feces.....	26.7

Clover infusion ration:

In urine.....	27.4
In feces.....	40.7

IV. The average daily nitrogen intake for the calves was as follows:

	<i>grams</i>
Calf 22.....	85.96
Calf 110.....	57.41
Calf 219.....	38.66
Calf 223.....	48.72

V. Average daily gain in body weight per gram of nitrogen retained.

Calf 22.....	28.5
Calf 219.....	38.4
Calf 110.....	22.8
Calf 223.....	30.2

## EXPERIMENT II

### *Introduction*

From our previous experiment<sup>5</sup> in feeding liquid beef blood as a source of animal protein, we deemed it worth while to continue the use of blood protein as a substitute for milk protein. As stated in our previous discussion and as must be evident to any feeder, liquid blood, as used in our experiment, is not a practical feed, owing to the difficulty of securing a sufficient quantity at regular intervals. This would be possible only in locations where large slaughter houses are located. The greatest objectionable feature in the use of liquid blood is its rapid deterioration, which makes it unfit for this purpose.

<sup>5</sup> Caldwell, R. E., Dairy Science, ii, 312.

To secure what seemed to us a practical combination, judging from results obtained from our previous feeding experiments with mixtures of grains and proteins from different sources, we selected for our mixture, ground corn, oil meal, and liquid beef blood. The proportions were corn meal, 8 parts, oil meal 1 part, and liquid blood 12 parts, corresponding approximately to 3 parts of dried blood. The proportion of corn meal and oil meal were suggested by work done at the Wisconsin Agricultural Experiment Station. It was found that when corn alone was fed the proteins were utilized to the extent of 24 per cent and oil meal alone 20 per cent, but when mixed in the proportion of corn protein 75 per cent and oil meal protein 25 per cent and fed, 40 per cent of these proteins was utilized in the formation of body protein. These grains are easily available and moderate in price, and the mucilaginous body in the oil meal serves an excellent purpose in keeping the mixture in suspension when mixed with warm water.

### *Purpose*

The purpose of this experiment was to determine the efficiency of this mixture of corn meal, oil meal, and liquid blood when prepared in a dry condition, which ultimately could be manufactured on a large scale and made a practical commercial feed for raising young calves.

### *Preparation of feed*

In preparing the mixture of corn meal, oil meal, and liquid blood for this feeding experiment particular attention was given to the drying. It is generally accepted that high heat, at the boiling point of water deteriorates or has a destructive influence on the activity of the unknown dietary factors, "vitamines." And no doubt, continued high heat, especially above the boiling point of water, causes the proteins to become less soluble and digestible in the alimentary tract. In order to avoid the effect of high heat, the mixture was dried on shallow tin pans at a temperature not above 140° to 160°F. The mixture was stirred

frequently. During the drying a warm blast of air was constantly forced over the mixture, this procedure hastening the drying and avoiding undue exposure. This drying was completed in 4 to 6 hours sufficiently to permit grinding. After drying the feed was ground fine enough to pass a forty mesh sieve. This feed, when mixed with water 160° to 180°F. and left standing for a short time, becomes gelatinous and can be diluted with water to the required consistency for feeding. The mixture of corn meal, oil meal and liquid blood as prepared above, we will designate in the future as "Purdue Calf Meal."

#### *Method of procedure*

Two Holstein grade male calves of about the same age were selected for this feeding trial. The calves weighed 119 to 129 pounds, age fourteen to sixteen days respectively. They were fed in feeding crates as in our previous experiment. Milk having been fed for two days it was replaced by the calf meal. This was gradually increased daily so that at the end of seven days the calves were fed exclusively on this meal. In addition to this prepared feed the calves were fed a dry mash consisting of ground corn 8 parts, and oil meal 1 part, in such quantity that the trough was clean of mash at each feeding. The calves had access to roughage of chopped corn fodder of excellent grade, of which they were allowed all they would eat. The uneaten portion, consisting of some husks and stocks was removed daily and fresh fodder supplied. A record was kept of all feeds fed and consumed.

#### *Nitrogen content of feeds*

	per cent N
Milk . . . . .	0.5
Blood mixture . . . . .	4.24
Dry mash . . . . .	1.98
Stover . . . . .	1.00

*Discussion of experiment II*

In this discussion it is our aim to show the efficiency of Purdue Calf Meal compared with that of liquid blood and milk.

The average daily gain of calf 22, fed on liquid blood, was 1.32 pounds, calves 219 and 110, receiving milk, made an average daily gain of 1.40 and 1.17 pounds respectively, while calves 1 and 2 receiving Purdue Calf Meal made an average daily gain of 0.96 and 0.94 pounds.

From the summary it is seen that the average daily nitrogen intake for calf 22 was 85.96 grams, and for calves 219 and 110 the average daily nitrogen intake was 38.66 and 57.47 grams respectively. If we consider the nitrogen retained for body growth of the different rations, we find that calf 22 retained 21.09 grams of nitrogen daily, calves, 219 and 110 retained 16.6 and 23.25 grams daily while calves 1 and 2 retained 19.58 and 21.81 grams of nitrogen daily. From these figures it appears that calf 22 received more nitrogen than the capacity for growth required. When the digestible nitrogen intake is greater than that required for growth, the excess is catabolized (that is, the living protoplasm breaks down complex substances into simpler components), the residual nitrogen appearing in the urine. This seems quite evident from the figures obtained in the distribution of the excreted nitrogen. Summary I and II. By comparing the gain in weight per gram of nitrogen intake we find a close relation with the exception of calf 219.

*Daily gain per gram of nitrogen retained*

Calf 22 .....	28.5
Calf 219 .....	38.4
Calf 110 .....	22.8
Calf 1 .....	22.2
Calf 2 .....	19.6

The figures show the nitrogen distribution of the calves fed different rations.

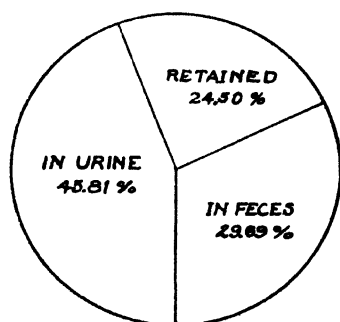


FIG. 1

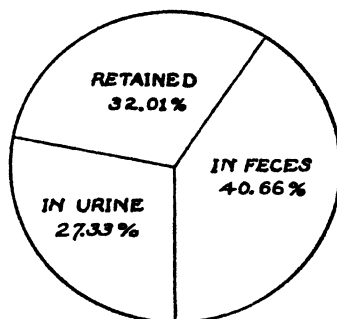


FIG. 3

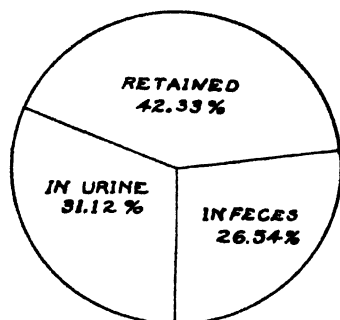


FIG. 2

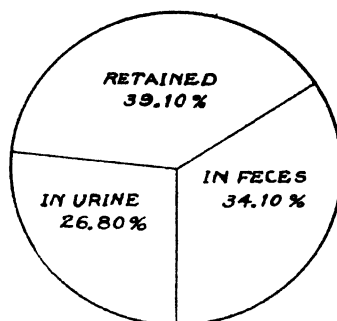


FIG. 4

Figure	No.	
1	22	Liquid blood ration
2	110	Milk ration (average)
	219	
3	223	Clover infusion ration
4	1	Purdue Calf Meal ration (average)
	2	

The circles represent the total nitrogen intake, and each sector shows nitrogen retained, excreted in feces and urine.

These figures together with the gain in weight per gram of nitrogen intake represent the efficiency of Purdue Calf Meal as a feed for young calves.

### *Summary of experiment II*

I. The following is the average daily gain in pounds for the two calves fed Purdue Calf Meal.

	pounds
Calf 1.....	0.96
Calf 2.....	0.94

II. The following per cent of nitrogen fed was retained.

	per cent
Calf 1.....	37.08
Calf 2.....	41.20

III. Of the intake nitrogen the excreted nitrogen was distributed as follows:

	per cent
Calf 1, in urine.....	27.5
Calf 1, in feces.....	35.5
Calf 2, in urine.....	28.1
Calf 2, in feces.....	32.7

IV. The average daily nitrogen intake for the calves was as follows:

	grams
Calf 1.....	52.92
Calf 2.....	52.94

V. Average daily gain in body weight per gram of nitrogen intake.

Calf 1.....	8.2
Calf 2.....	8.1

VI. Average daily gain in body weight per gram of nitrogen retained.

Calf 1.....	22.2
Calf 2.....	19.6



A GROUP OF CALVES FED PURDUE CALF MEAL



ONE OF THE EXPERIMENTAL CALVES FED PURDUE CALF MEAL,  
AGE 5 MONTHS



EXPERIMENT III<sup>6</sup>*Introduction*

To follow on our experiments for the past several years in feeding young calves in crates different mixtures of feeds, and because of the comparatively uniform results of the feeding of blood meal, we were led to try this prepared calf feed with a large number of calves. It is evident that crate feeding can not give the same results as when calves are fed in stalls or when conditions are more favorable for development and well-being of animals. In crate feeding the calves are deprived of two of the essentials of life, exercise and freedom. In order to test the efficiency of any mixed feed and draw conclusions as to its relative value, if it is to become a commercial product, it must be fed to a larger number of animals having more natural environments, such as out door exercise and pasture.

*Purpose*

From the practical feeder's point of view, it seemed highly desirable to know the development and well-being of the animals of this experimental feeding after the earlier stages or critical period of the young animal's life. To test the efficiency of this feed after the first seventy to seventy-four days' feeding, the feeding was continued seventy to seventy-seven days longer, making a total of one hundred and forty to one hundred and fifty-one days. To compare the efficiency of Purdue Calf Meal with that of other feeds, two lots of calves of approximately like age, weight and general condition were fed on milk, one lot on skim milk and one lot on whole milk. It was also our purpose to test the performance of the three lots of calves after the expiration of the second feeding period when they were put on blue grass pasture. The three lots of calves during this

<sup>6</sup> Acknowledgment is due to Prof. O. E. Reed, chief of the dairy department, for his interest in these experiments and for the extra means and privileges which made the third feeding experiment possible. Also to Swift and Company of Chicago, Illinois, for their kindness in preparing the calf meal especially for us as used in our last feeding experiment.

period had the same natural advantages. The pasturing was to be continued until the calves were one year old.

### *Preparation of feed*

Owing to the larger number of calves fed in this experiment and the limited facilities in the laboratory, the calf meal was manufactured by Swift and Company in quantities sufficient to complete the experiment. The same proportions of corn meal 8 parts, oil meal 1 part and liquid blood 12 parts were used; in addition 1 per cent of bone meal was added, the drying was done at a temperature as near as possible to that of our previous feeding experiment, when the calf meal was made in the laboratory. After drying, the meal was ground to pass a 30 to 40 mesh sieve.

### *Number of calves used in the following experimental feeding*

Thirty-six grade Holstein calves were purchased from the stock yards at Chicago for use in this experiment. These calves ranged in age from ten to twenty days as far as could be determined. They were remarkably uniform in vigor and general appearance, weighing from 85 to 126 pounds. The thirty-six calves were divided into three lots. Lot I and II received whole milk and skim milk respectively, lot III received Purdue Calf Meal. The total weight of each lot of 12 calves was: Lot I, 1205; lot II, 1180; and lot III, 1201 pounds.

### *Feeding and stabling*

*Feeding.* Each calf was taught to drink from a pail on arriving from the stock yards. They were all fed on whole milk for ten days. Lot II was gradually changed to skim milk. Lot III was changed to Purdue Calf Meal by gradually increasing the quantity of Calf Meal. Ten days were taken to make the change from whole milk to skim milk and calf meal. Lots I and II fed on whole milk and skim milk, received milk in proportion to their live weight, 10 pounds of milk daily per 100 pounds weight.

This ratio was continued until calves weighed 200 pounds and were receiving 20 pounds of milk. No increase in the quantity of milk fed was made after the calf weighed 200 pounds. Lot III fed on calf meal, received a definite quantity of meal per day based on proportionate amount of nitrogen contained in milk and this was continued throughout the feeding period. All calves were fed a grain ration composed of equal parts of ground corn and oats. The ground grain was weighed for each lot of calves daily and a record kept of the amount eaten. The calves had access to good clover hay which was supplied in sufficient quantity to meet their wants and was not wasted. They were watered at regular intervals.

#### *Method of feeding calf meal*

Several preliminary trials were made concerning the quantity of calf meal which a young calf could eat without causing digestive disturbance, scours and consequent failure to eat.

The first trial of feeding the required amount to a lot of calves was based on the nitrogen content of the milk fed. Knowing the percentage of nitrogen in milk and that of the feed, a nitrogen equivalent of the feed can be calculated corresponding to the quantity of milk. In our first trial the digestibility of milk and meal was taken into consideration, and a proportionately large quantity of meal was fed. We soon realized that the calves were over-fed. They were all subject to scours and indigestion which was manifested by the condition of the feces and their refusal to eat. On reducing the quantity fed to a much smaller amount they soon regained their normal condition. In our next trial of feeding the meal the amount fed was based on the nitrogen equivalent of milk.

The calculations were based on the percentage of nitrogen in milk at 0.5 per cent and that nitrogen of Purdue Calf Meal at 5.0 per cent. The milk-fed calves received 10 pounds of milk daily for each hundred pounds of live weight or 120 pounds of milk for twelve calves weighing 100 pounds each (or per lot of total weight of 1200 pounds live weight).

120.0 pounds of milk (N. O. 5 per cent)	= 280 grams of nitrogen
12.1 pounds of Purdue Calf Meal	= 275 grams of nitrogen

This proportion of calf meal was fed with a *decided* improvement. For very young calves it was found to be too much, causing scouring among the weaker calves but no apparent indigestion was shown by the appearance of the feces.

Another feeding trial was made, reducing the quantity of the meal so that the nitrogen from this source was 80 per cent of that contained in the milk. Feeding this proportion of calf meal, that is 9.6 pounds of meal to 120 pounds of milk proved to be the safe and correct amount to feed.

This quantity, 9.6 pounds of calf meal to 1200 pounds of live weight, was fed to lot III during the feeding period. We believe this proportion can be safely increased to 90 per cent of that of milk nitrogen after the calf has reached the age of ninety or one hundred days. Much will depend on the breed and individual vigor of the calves. The feeder must be the final judge. A safe criterion concerning the feeding is the physical condition of the feces when compared with the feces of a normal milk-fed calf. The above quantity, 9.6 pounds of calf meal, is the maximum quantity which we were able to feed during the feeding period.

#### *Preparation of feed and quantity of calf meal used for feeding*

If the total weight of a lot of calves is known, it is a simple matter to calculate the amount of meal required for the lot. To feed each calf its proper proportion based on its weight, the weight of each calf must be known. To prepare the calf meal for feeding, the meal is weighed and placed into a container of sufficient capacity to hold the mixture when properly diluted. To the meal in the container about four times its weight of warm water at 160° to 180°F. is added and well mixed. This is left standing for one to two hours and then diluted to a consistency for the calves to drink. The feed is prepared twice daily and fed at blood heat, about 98° to 99°F.

When feeding a lot of calves the diluted calf meal, the weight of each calf must be taken into consideration to properly apportion the feed on the basis of live weight. It is convenient to dilute the mixture to a total weight which is divisible by the number of calves. In our feeding where 12 calves were fed, the lot weighing 1200 pounds, 4.8 pounds of calf meal was prepared as previously directed and diluted to 48 pounds per feed. This would require 4 pounds of the diluted feed to each 100 pounds of live weight.

*Table showing the quantity of the liquid prepared feed fed to a lot of 12 calves*

NUMBER OF CALF	WEIGHT OF CALF	POUNDS OF PREPARED FEED AT EACH FEED
1	85	$0.85 \times 4 = 3.40$
2	88	$0.88 \times 4 = 3.52$
3	126	$1.26 \times 4 = 5.04$
4	93	$0.93 \times 4 = 3.72$
5	96	$0.96 \times 4 = 3.84$
6	123	$1.23 \times 4 = 4.92$
7	100	$1.00 \times 4 = 4.00$
8	100	$1.00 \times 4 = 4.00$
9	114	$1.14 \times 4 = 4.56$
10	95	$0.95 \times 4 = 3.80$
11	87	$0.87 \times 4 = 3.48$
12	94	$0.94 \times 4 = 3.76$

Total weight, 1201; weight of feed fed, 48.04.

When the total weight of the lot of calves reaches 1400 pounds it would require 0.8 pound more of the meal per feed for the twelve calves and the liquid mixture made up to 56 pounds. Again multiply the weight of each calf at this weighing, expressed in hundredths, by 4; this will give the required amount of liquid feed to feed each calf. The above mentioned dilution and proportioning has proved successful in our feeding experiments. It must not be inferred that the rate of dilution must be strictly adhered to. Each feeder must be guided by the behavior of the calves; for some calves, or at different periods of feeding, the consistency of the liquid calf feed would require to be changed.

*Weighing*

The calves of these lots were weighed at intervals of seven days. The weighings were made at about the same time each weigh day. This was always done in the forenoon after the morning feeding.

*Pasture*

All calves had access to blue grass pasture in season when weather permitted them to be turned out. They also had access to clover hay when out in the open. The hay was placed in an open rack sufficiently large to accommodate all calves without crowding.

*Discussion of experiment III*

In this experiment we endeavored to show the efficiency of Purdue Calf Meal when fed to a larger number of calves, eliminating individual peculiarities to a much greater extent. In the absence of a chemical analysis of the excreta only a comparison of the nitrogen intake and gain in body weight can be made which was deemed sufficient for the purpose of this experiment.

In summary III it will be seen that the average daily nitrogen intake for the three lots of calves was rather uniform. During the first feeding period the grams of nitrogen intake were for lots I, II, III, 52.1, 54.7 and 52.5.<sup>7</sup> For the second period the nitrogen intake was for lots I, II and III, 85.4, 90.3, and 94.3 grams daily.

If we compare the gain in body weight per gram of nitrogen intake from the different sources, we find the average daily gain in weight for the entire feeding period to be as follows:

	<i>Grams daily gain</i>
Whole milk ration .....	13.5
Skim milk ration .....	11.0
Purdue Calf Meal .....	7.3

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<sup>7</sup> By nitrogen intake we mean the nitrogen derived from the milk, calf meal and mash. Clover hay was fed to all lots, but no record kept and may be considered a constant.

It will be noted that the skim milk and calf meal ration produced a gain in weight per gram of nitrogen in this experiment approximately the same as in our previous experiments.

During this feeding experiment the calves fed Purdue Calf Meal received less than 80 per cent as much nitrogen from the calf meal as the milk fed calves received from milk. The balance of the nitrogen intake was derived from the mash. The effect of the nitrogen derived from mash in the growth of the calf is shown by comparing the gain in weight of the three lots during the first and second period. During the earlier stages of feeding, the proteins derived from the mash were not utilized for growth as during the second period of feeding.

The three lots of calves, fed whole milk, skim milk, and calf meal received the following average daily quantity of nitrogen from these sources.

<i>Period I</i>		
<i>Lot I</i>	<i>Lot II</i>	<i>Lot III</i>
34.9 grams of nitrogen	35.5 grams of nitrogen	19.3 grams of nitrogen
<i>Period II</i>		
45.0 grams of nitrogen	46.1 grams of nitrogen	35.2 grams of nitrogen

If we base the gain in body weight on the nitrogen obtained from whole milk, skim milk and calf meal, we find a close relation of gain in weight for the three lots of calves.

<i>Average daily gain in weight per gram of nitrogen</i>		
<i>Period I</i>		
<i>Lot I</i>	<i>Lot II</i>	<i>Lot III</i>
Whole milk, 22.3 grams	Skim milk, 18.0 grams	Calf meal, 19.4 grams
<i>Period II</i>		
Whole milk, 22.9 grams	Skim milk, 20.7 grams	Calf meal, 20.0 grams

From the above figures it seems that in all Lots the proteins derived from animal origin, that is, milk and blood, are the principal factors in promoting growth and approximately equal in efficiency gram for gram. The nitrogen derived from the mash must have been utilized to a certain extent in the production of growth but not in proportion to the quantity consumed.

The energy required to digest proteins and carbohydrates from grains must be taken into consideration in feeding young calves. This is an important factor especially where the energy required to transform the food into an assimilative condition may nearly equal that required for maintenance. This is shown in feeding of the calf meal during the first and second periods. During the first period of 70 days the calves made an average daily gain of only .83 pounds, and in the second period an average daily gain of 1.53 pounds. This emphasizes the ability of the calves to utilize the feed much more efficiently in the second period after the calves reached the age of eighty to ninety days than in the first period. This was further emphasized during pasturing after the experimental feeding, when the calves from the three lots made the same average daily gain during the six months when on pasture.

### *Summary of experiment III*

The following represents the average daily gain in weight for the three feeds, whole milk, skim milk and Purdue Calf Meal.

#### I. Gain during first period:

	<i>pounds</i>
Whole milk ration (Lot I) . . . . .	1.72
Skim milk ration (Lot II) . . . . .	1.41
Purdue Calf Meal ration (Lot III) . . . . .	0.83

#### II. Gain during second period:

	<i>pounds</i>
Whole milk ration (Lot I) . . . . .	2.26
Skim milk ration (Lot II) . . . . .	2.06
Purdue Calf Meal ration (Lot III) . . . . .	1.53

#### III. Average daily grams of nitrogen consumed during first feeding period:

	<i>grams</i>
Whole milk ration (Lot I) . . . . .	52.1
Skim milk ration (Lot II) . . . . .	54.7
Purdue Calf Meal ration (Lot III) . . . . .	52.5

#### IV. Average daily grams of nitrogen consumed during second feeding period:



	<i>grams</i>
Whole milk ration (Lot I) . . . . .	85.4
Skim milk ration (Lot II) . . . . .	90.3
Purdue Calf Meal ration (Lot III) . . . . .	94.6

V. Average daily gain in body weight per gram of nitrogen consumed:

*First period*

	<i>grams</i>
Whole milk ration (Lot I) . . . . .	14.9
Skim milk ration (Lot II) . . . . .	11.7
Purdue Calf Meal ration (Lot III) . . . . .	7.2

VI. Average daily gain in body weight per gram of nitrogen consumed:

*Second period*

	<i>grams</i>
Whole milk ration (Lot I) . . . . .	12.1
Skim milk ration (Lot II) . . . . .	10.3
Purdue Calf Meal ration (Lot III) . . . . .	7.4

VII. Average daily gain in body weight for the entire feeding:

	<i>pounds</i>	<i>days</i>
Whole milk ration . . . . .	1.91	140
Skim milk ration . . . . .	1.73	140
Purdue Calf Meal ration . . . . .	1.18	151

VIII. Gain in weight of the different lots when put on pasture: After the 140 days feeding, the calves were put on blue grass pasture, and during the six months the following average daily gains were made by each calf of the different lots.

	<i>pounds</i>
Whole milk ration (Lot I) . . . . .	1.16
Skim milk ration (Lot II) . . . . .	1.24
Purdue Calf Meal ration (Lot III) . . . . .	1.18

IX. At the age of eighteen months the average weight per calf was for:

	<i>pounds</i>
Whole milk ration (Lot I) . . . . .	582
Skim milk ration (Lot II) . . . . .	594
Purdue Calf Meal ration (Lot III) . . . . .	475

The difference in weight of the calves fed calf meal and milk occurred principally during the first period of seventy to ninety days.

## SUMMARY

1. Beef blood when combined with corn and oil meal in proportion of 8 parts corn meal, 1 part oil meal and 12 parts beef blood furnishes a reasonably successful milk substitute for feeding young calves.

2. The development of the calves when on pasture indicates that the feeding of Purdue Calf meal seems not to have impaired or stunted the growth impetus, as the gain in weight of calves fed Purdue Calf Meal was equal to the gain in weight of the milk fed calves while on pasture.

# THE WATER BUFFALO AS A DAIRY ANIMAL

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## HISTORY OF THE WATER BUFFALO

The original home of the water buffalo (*Bubalus bubalis* Lyd.), is not definitely known. However, credit for the first domestication of this class of bovine is probably due to the people of northern India where today the undomesticated form still exists, although in small numbers.

At an early date, the water buffalo was domesticated and had reached what is now southern and southwestern China. Chinese literature refers to it as having been used as a draft animal and as a source of beef for centuries by the farmers of southern China.

## DISTRIBUTION TODAY

Today the water buffalo, as a domesticated animal, is common in practically all of India, Siam, Straits Settlements, Indo-China, China as far north as two hundred miles north of Shanghai, the Philippine and Hawaiian Islands, and other islands of the Pacific Ocean. From the Hawaiian group, they jumped across to Brazil in South America. At an early date, as early as 600 A.D., according to some writers, they reached southern Europe, where today, in Italy, they may be found plowing the small fields for the Neapolitan farmers. No attempt has ever been made to introduce them to the United States. Great numbers are kept on the bottom land of the Danube, the Theiss, and the Drave in Austria-Hungary, and in Italy on the plains lying to the north and east of Naples. Indian dairy breeds have been introduced to Trinidad, where they are used as draft animals and are both worked and milked by Hindu coolies.

Wild buffaloes are still to be found in India, in the grassy jungles along the large rivers of that land, and in the open marshy prairies, the finest specimens being in Assam and Burma, and in the Straits Settlements, Java, Ceylon, Sumatra and Borneo (1). A wild form, different from the Asiatic species, exists in parts of Africa. The African species is exceedingly dangerous and has never been tamed.

#### GENERAL DESCRIPTION OF THE WATER BUFFALO

When compared in size with other classes of animals in the bovine group, the water buffalo is a relatively large animal. The average size for mature animals of the beef-draft type, which is the type common in China and in the Islands of the Pacific, is between 800 and 1400 pounds. In height, they vary from 46 to 50 inches at the withers. The dairy breeds of northern and central India, especially those in the region of Delhi in the Bombay presidency, is much larger in size (2). A herd of thirty cows of this breed in Kowloon, near Victoria, Hongkong, which were studied by the writer in 1918, varied in height from 56 to 60 inches at the withers. The weight of this breed is considerably more than that of the beef-draft type common in China. Animals in ordinary flesh, in which condition there is no superfluous fat on the body, weigh from 1500 to 2000 pounds. Other minor dairy breeds of India are the Jafferabad, from the west, and Deccani and Nagpur breeds from the central part of India (2).

In color, the water buffalo is a dark grey, or almost black, with now and then white markings on their face and legs. Pure white albinos are now and then seen, especially in parts of Tonkin province in southwestern China, where they are in special favor because of their supposed immunity to rinderpest.

The skin of the water buffalo, like that of the pig, has practically no sweat glands, and, for this reason, the buffalo likes to wallow in mud or water on a hot day, and thereby it gets its name, "water buffalo." There are also apparently no oil glands in the skin of the buffalo. The mature animal has very little hair on its body. The sides, back, and thighs of the animal are

practically bare. The shoulders and knees have the most hair, where it is about 2 to 3 inches long, and very thin. The buffalo calves are born with a rather heavy coat of long, brown hair all over the body, which gradually disappears after one year of age is reached. The horns of the buffalo are characteristic, although they vary in size and shape, with different breeds. In general, those of the beef-draft type are long, thick and somewhat triangular at the base, fairly sharp at the tip, with distinct grooves on the upper surface. They are directed out, back and somewhat upward from the head, and curve inward at the tips. The length of the horns, measured on the outside of the curve, is usually about 2 feet, although they may vary from 12 inches to 4 feet or more. The movements of the water buffalo are rather slow and unwieldy, similar to that of the elephant; in fact, a casual view of a moving herd of buffalos suggests a roving band of elephants.

The udders of both the beef-draft types of China and dairy breeds of India are rather small, but well shaped, with well developed teats. The beef-draft animals have a wide back and loin, with short legs and a large barrel. The body, as a rule, is short and compact, and well-built for draft purposes. The croup is decidedly sloping. They fatten readily, and buffalo beef from young animals is similar to that of the *Bos taurus* or *Bos Indicus*, although it is darker in color.

There are a number of striking differences in the Delhi dairy breed of buffalo from India, and the beef-draft type mentioned above. The horns of the Delhi breed rise from the head in an upward and backward direction, and then curl over the head like the horns of a ram, and, for this reason, in the Philippine Islands they are called "ram's horn" buffalos. The body is lean and angular. The teats are well placed and of rather large size, usually somewhat constricted at their attachment to the udder. They have a very large frontal bone in the head, and small, wall eyes. Although rather fierce looking on account of their large horns, prominent forehead and glassy eyes, the Delhi buffalo are very docile and easily managed, by Europeans as well as by Indians. The beef-draft type of southern China, on the

other hand, while easily managed by the natives of that land, dislike strangers, especially Europeans, who find it difficult to manage.

The gestation period of the water buffalo varies from ten to eleven months. The calves generally weigh from 60 to 80 pounds at birth.

#### USES OF THE WATER BUFFALO

The principal use of the water buffalo in China and the Pacific Islands is for draft purposes. Surplus animals, or animals too old for good use in the fields are fattened and slaughtered for beef.

They possess great strength, although they are not fast in getting over the ground when compared with draft horses. It was found by Gandencio Evaristo (3) at Los Banos, Philippine Islands, that, in fairly light soil, and on a rainy or cloudy day, a buffalo could plow 5000 to 7000 square meters a day.

For uplands, where water is not available close at hand, the buffalo is of little use, except where work is limited to the early morning or late afternoon. This is because it is unable to stand the heat of midday without being kept constantly cooled with water on its body surface.

In India, where the religion of the natives largely prohibits the use of the flesh of animals for food, the buffalo is used for draft and dairy purposes only. When used for draft purposes, they are used singly, single-tandem, or two are yoked together and driven in the way which oxen are generally used. As a rule, in China only one buffalo is used on a plow. The plows cut a furrow slice from six to eight inches wide and two to five deep.

#### THE CHINESE DRAFT WATER BUFFALO AS A POSSIBLE DAIRY ANIMAL

No dairy breed of water buffalos has yet been developed in China. This is probably largely due to the fact that, until the advent of the Europeans in China, the Chinese did not consider

milk from cattle fit for human food. It is strange that this opinion should have so generally prevailed, for the Tibetans on the west and the Mongolians on the north have, from time immemorial, been users of dairy products in the form of milk, butter and cheese, made from cow's, goat's, yack's or mare's milk. It is only during the present generation that the Chinese have begun to use milk to any extent, and its use today is confined chiefly to the regions where the Chinese have come in contact with Europeans and western methods.

The fact that the milk of the Chinese buffalo cow is very rich in fat, and palatable, has been known in a general way for many years, but it is only some fifteen years ago that the first water buffalo dairy was started in Canton.

#### STUDY OF THE WATER BUFFALO AT CANTON CHRISTIAN COLLEGE

From the fall of 1916 to the summer of 1919, considerable data in regard to the Chinese water buffalo as a dairy animal was collected by the writer while on the agricultural staff of the Canton Christian College, Canton, China. Some of this data has been published in various journals and bulletins (4).

#### EXPLANATION OF MILK ANALYSES AND RECORDS

The following tables give the analysis of milk and production records of cows for which we have records for entire lactation periods.

Butter fat analyses were made twice a month with a Babcock centrifugal tester. The morning and evening milk was tested separately. The averages of the two tests was taken as the average test for the month. The milk for twenty-four hours was weighed twice a month. The average amount of milk for the two weighings was taken as the average daily production for the month.

The total solids (consisting of the fat, sugar, proteids, and ash) were found by evaporating a weighed sample of milk in a steam bath until the weight became constant. The ash was determined by heating in a crucible over a gas flame until the weight

became constant. The proteids were determined by the Kjeldahl method as described by Hawk in his Practical Physiological Chemistry, fourth edition, pages 410 and 483. The sugar was found by subtracting the sum of the ash, proteids and fat from the total solids. The percentages in each case were found by dividing the weight of the final product by the weight of the sample of milk analyzed.

Analysis and records of buffalo milk are all with cows in the college dairy.

The buffalo cows were fed a grain ration consisting of two pounds wheat bran and one pound rice chop. Each cow was fed about a pound of this mixed feed a day for each pound of milk given daily. The rice was fed cooked. The grain was fed separately to each cow twice a day. About  $1\frac{1}{2}$  ounces of salt was fed daily to the cows. The salt was mixed with the grain. About 40 pounds of water was mixed with the rice chop and bran at each feeding, making a very wet feed, the cows drinking it down rather than eating it. (This is the usual method of feeding grain to cattle in China.) The cows were fed all they could consume four times a day of a mixture of green cut grass, which amounted to from 40 to 80 pounds a day per cow.

Table 1 brings out a number of important facts about the milking qualities of the water buffalo in China. The average percentage of fat for the six cows for which records throughout a lactation period are available, was 11.25. The average amount of milk produced was 2293.3 pounds and the average amount of fat was 250.85 pounds, the highest being 326.31 pounds for cow 54. Another important fact brought out in these records, as shown in table 1, is the long lactation periods of the buffalo. As a rule, the cows in the college herd were milked for from eight to twelve months, depending on how soon after freshening they were bred. They are usually bred three months after freshening. When fresh, they give from 5 to 15 pounds a day, which diminishes very gradually throughout the long lactation period.



The production capacity of the cows in this experiment is striking when we consider the fact that the ten cows used were ordinary village cows of the beef-draft type, whose ancestors had never been used for dairy purposes.

TABLE 1

*Analysis of milk; length of lactation period, amount of milk, amount of fat, and percentage of fat of the Chinese water buffalo milk*

COW NUMBER	LENGTH OF LACTATION PERIOD	TOTAL AMOUNT OF MILK	TOTAL AMOUNT OF FAT	AVERAGE PERCENTAGE OF FAT
	<i>days</i>	<i>pounds</i>	<i>pounds</i>	
51	330	2863.4	278.22	9.81
53	277	1909.0	211.93	11.10
54	365	2644.5	326.31	12.33
55	253	1871.5	227.38	12.15
60	273	1849.0	203.68	11.02
63	325	2322.4	257.60	11.09

TABLE 2

*Complete analyses of buffalo milk: The samples analysed were herd samples taken from twelve cows in the college dairy in November, 1917. The milking in the morning was begun at four, and in the afternoon at two o'clock*

SAMPLE NUMBER	FAT	ASH	PROTEIN	SUGAR	TOTAL SOLIDS	WATER
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1. Morning milk.....	11.00	0.94	6.04	4.00	21.98	77.02
2. Afternoon milk.....	12.80	0.90	6.10	3.57	23.37	76.65
3. Afternoon milk.....	13.00	0.71	5.71	3.32	22.74	76.98
4. Afternoon milk.....	13.63	0.74	5.94	3.57	23.92	77.08
5. Afternoon milk.....	12.10	0.90	6.14	3.83	22.87	77.04
6. Afternoon milk.....	14.00	0.92	6.42	3.87	25.21	74.79
7. Morning milk.....	11.50	0.95	5.80	3.70	21.95	77.05
8. Morning milk.....	12.00	0.94	6.00	3.60	22.54	77.45
9. Morning milk.....	12.34	1.04	6.28	3.71	23.10	76.63
10. Morning milk.....	12.20	0.77	5.90	4.23	23.02	76.98
Averages.....	12.46	0.89	6.03	3.74	23.29	76.89

As seen by table 2, the milk contains a great deal more proteids, sugar, somewhat more ash, and nearly twice as much solids as does European cow's milk.

ANALYSIS AND PRODUCTION OF THE DELHI (INDIAN) DAIRY  
BUFFALOS

In the region of Hongkong, there are a number of Delhi dairy water buffalo herds owned and managed by Indians. Dr. Adam Gibson, the Colonial Veterinarian, of Hongkong, who is also milk inspector for that territory, has informed the writer that the per cent of fat in the milk of this breed is similar to that of the Chinese water buffalo. According to British authorities in India, this breed in India commonly gives from 30 to 50 pounds of milk a day (3).

In January, 1919, the writer visited an Indian water buffalo dairy in Kowloon, which at that time had a total of twenty cows giving milk. The average production for the twenty cows was 15 pounds. Some of the cows had recently freshened, while others were in the seventh and eighth month of the lactation period. The feed at that time consisted of dry rice straw for roughness, and for concentrates they received a small amount of cooked rice chop and wheat bran. The cows were crowded into a dark, damp, poorly ventilated and dirty barn. With proper care, and the addition of succulent feed in the ration, the production of this herd could no doubt easily be doubled.

CHARACTERISTICS OF THE BUFFALO MILK OTHER THAN THAT  
SHOWN BY ANALYSES

Water buffalo milk is pure white in color. Butter made from the milk is also pure white. It is wholesome and very palatable when produced under sanitary conditions. Students and teachers (both European and Chinese) at the Canton Christian College prefer water buffalo to European cow's milk. On account of it having a high per cent of solids, it has a richer flavor, and European cow's milk seems thin and watery after drinking buffalo milk.

## DISEASES OF THE WATER BUFFALO

Among water buffalos, as well as among the cattle of the Orient, probably the most common disease is a contagious disease known as rinderpest. The disease is somewhat like the chronic form of hog cholera, in that it is usually accompanied with fever and causes lesions in the inner lining of the intestines, but it is not as fatal as is cholera among hogs. Fortunately, a method of immunizing with anti-rinderpest serum has been worked out by veterinarians in the Bureau of Agriculture of the Philippines which is now being successfully applied in most parts of the Philippines, and used to some extent in India and Hongkong.

Tick fever, commonly known in the United States as Texas fever, after the region in which it is most common in the United States, is prevalent in South China. The disease is fatal to cattle imported from tick-free regions. Fortunately ticks do not travel far and breed only in grassy places. European cattle, in southern China, are usually kept in barns and in dry lots and suffer very little from the tick. The tick fever seems to have little or no effect on the water buffalos. They are apparently very highly resistant to the disease.

## TUBERCULOSIS

According to Dr. Heanley of the Hongkong Bacteriological and Vaccine Laboratory, tuberculosis has never been found among the buffalo of south China, and in thirteen years' inspection of animals and carcasses in the Hongkong government slaughter house, where all animals slaughtered for food are inspected by government inspectors, only two cases in the humped cattle have come to notice. Both cases were bullocks. The disease is as common among European cattle as it is in America. Dr. Gibson, who has inspected the carcasses of buffalos slaughtered in Hongkong for the past thirteen years, at the rate of several hundred per week, confirms Dr. Heanley's report in stating that he has never seen tuberculosis lesions in the water buffalo.

Dr. M. Prettner, made a careful study of the resisting power of the water buffalo against experimental tuberculosis (5). Only when he injected tubercular material a number of times in the same buffalo, in amounts many times that required to induce tuberculosis in a cow or goat, could he produce the disease in the buffalo. In fact, even then, upon post-mortem, it was found that the organisms were found only in an inflamed area around the point of insertion of the tubercular material. The buffalos also had to be kept in an underfed and run-down condition in order to produce this slight infection. He reports that among five thousand buffalos slaughtered for meat, no case of tuberculosis was observed.

#### THE BUFFALO COMPARED WITH THE EUROPEAN COW AS A DAIRY ANIMAL

There are a number of commendable features in the use of the buffalo cow as a dairy animal. The amount of butter fat in milk in the better buffalo cows of China (see production tables) is not insignificant when we consider that there has been no breeding for production. Ordinary cows give more than two thousand pounds of milk and 150 to 300 or more pounds of fat a year. The "ram's horn" buffalo from India rank with the best breeds of modern dairy cattle, both in production of milk and total butter fat. The fact that the buffalo has no sweat or oil glands in the skin makes it an easy animal to keep clean. The scant hair on the body affords a poor hiding place for lice, and they can be easily detected and gotten rid of. The absence of tuberculosis among buffalos, and their resistance to the tick fever adds to their value as dairy cows. It is the writer's opinion that in regions where the water buffalo is thriving, the breeding of the native buffalo for production, and the importation of Indian dairy buffalos should be encouraged in every way possible, rather than encourage the importation and breeding of European cows, because of the danger from tuberculosis in the European cows.

## OBJECTIONS TO THE BUFFALOS FOR DAIRY PURPOSES

In China, one of the chief reasons why so few buffalo cows are used for dairy purposes is no doubt because of the fact that they give but little milk, and that, while it contains about three and one-half times as much fat, and nearly twice as much total solids as does European cow's milk, it usually sells for the same price as the latter. However, as soon as the public knows the value of buffalo milk, it should command a much higher price than at present. Also the fact that cows which have not been especially bred for milk production, but simply selected from among ordinary buffalo herds, produce as much as 10 pounds of milk a day for several months, promises individuals giving a much larger amount of milk, with a few generations of selection and breeding for dairy purposes.

In warm weather, buffalo cows need a bath twice a day in order to keep at their best. It is the custom for them to be driven for about one-half hour each day to canals or ponds, which are not always clean for this wetting. To overcome this objection, tanks might be constructed in which clean water could be kept for their bath, or they may be wet twice a day by pouring clean water over them with buckets or with a hose where water pressure can be secured.

## THE FUTURE OF THE BUFFALO IN DAIRYING

In India the buffalo is the chief source of milk where it is not only competing with good native breeds of the "humped" variety, of cattle but also with modern breeds of European dairy cattle.

There is a strong prejudice among Europeans in India against the use of buffalo milk because of the unsanitary methods under which it is frequently produced. In much of India, the buffalo is a scavenger, subsisting largely on not only the refuse of the kitchen, but also on horse dung, and even on human waste. Naturally the flavor of milk produced from such feed has an uninviting odor and flavor. With the education of the village farmers in better feeding methods, this condition will no doubt be improved.

It is the opinion of the writer that the water buffalo is bound to become an important dairy animal in the southern half of China as well as an important source of milk for the four hundred million people of that vast country. Unlike the Indians, the Chinese have not been users of milk in the past, but are rapidly taking to the use of this beverage through the example set by Europeans. With a few generations of intelligent selection and breeding among the beef-draft buffalo of China, there will be developed a dairy breed of high producing ability. The possibility of this is shown at the Canton Christian College by the fact that with no breeding or selection cows from an ordinary village herd, whose ancestors had never been milked, gave two thousand and more pounds of milk containing as much as two hundred sixty pounds of fat in less than one year.

The history of both the dairy and beef breeds of European cattle in most parts of the Philippine Islands has been little more than a keen disappointment and failure. The climate and diseases of most parts of the Islands are such that European cattle, as a rule, quickly succumb to diseases or degenerate from generation to generation. Two years ago, the Philippine legislature appropriated a large sum of money for the improvement of live stock in the Island. Very little of this money was used for importing more European cattle, but on the other hand, 200,000 pesos were set aside by the Bureau of Agriculture and the College of Agriculture for the importation of the Delhi dairy buffalos from northern India.

It is difficult to prophesy as to the future of the buffalo in the United States, if it is to have a future in this country. There is no doubt but that the buffalo would thrive in most parts of the south as far north as the southern part of Oklahoma. Most regions further north would probably be too cold. The swamps and marshes of Florida, Louisiana, and Mississippi should be especially adapted to buffalo production.

The fact that the buffalo is free from tuberculosis, as well as an excellent producer of milk and butter fat, may result in an attempt to introduce certain of the more distinct dairy breeds to the southern states. However, extreme care will need to be

used in preventing the introduction with the animals of diseases which have not yet found a footing in this country. Further study of the buffalo in its native land should be made in order to become better acquainted with its diseases and other problems connected with buffalo raising before the attempt to raise buffalos in America is made.

## REFERENCES

- (1) BAILEY: Cyclopedia of American Agriculture, iii, 292.
- (2) HEWLETT: Breeds of Indian Cattle, Bombay Presidency, pp. 32-33, 40.
- (3) EVARISTO: Philippine Agriculturist and Forester, iv, 131.
- (4) LEVINE: Notes on Farm Animals and Animal Industries in China, Canton Christian College (Canton, China), Bul. 23 (1919), pp. 35-47.  
LEVINE AND CADBURY: A Study of Milk Produced in Kwangtung, Canton Christian College, Bul. 18 (1917).  
LEVINE: Analysis of water buffalo milk in South China. Philippine Journal of Science, July, 1919.  
LEVINE: The water buffalo as a dairy animal. Journal of Heredity, February, 1920.
- (5) PRETTNER: The resistance of the buffalo against experimental tuberculosis. Centralblatt für bakteriologie u. parasitenkunde. 1 abt. Orig., xxxi, 681-686.

# THE RELATIVE VALUE OF THE METHYLENE BLUE REDUCTION TEST, THE BROMTHYMOL BLUE TEST, AND THE BROMCRESOL PURPLE TEST IN DETERMINING THE KEEPING QUALITY OF MILK<sup>1</sup>

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The various factors that determine the quality of milk have been presented in papers published in previous numbers of this Journal (1) (2). In the latter paper it was suggested that the consumer is interested in the richness, safety, cleanliness, and sweetness of the supply. The healthfulness and keeping quality are dependent on the bacterial content of the milk. The extent to which foreign matter has been introduced into the milk is also reflected in some degree by its bacterial content. The bacterial flora of every sample of milk is very complex, for many kinds of organisms are introduced from varied sources. Those which are unable to grow in milk can be of no importance in influencing its keeping quality. The detection of these types, however, may be of value in judging the quality of the milk, since they give some indication as to the extent to which it has been contaminated with foreign matter.

The forms that can grow in milk influence its keeping quality in varying degrees, the extent of which is determined by the temperature at which they grow most rapidly and by the nature of their by-products. If they are unable to grow at the temperatures at which milk is handled commercially, they can have no influence on its keeping quality. Again, if the chief by-products of an organism are carbon dioxide and hydrogen, as is apparently the case with certain of the bacteria of the *B. lactis*

<sup>1</sup> Published with the Permission of the Director of the Wisconsin Experiment Station.



aerogenes group, the organism will be a minor factor in determining keeping quality as compared to one that grows equally well in milk, and whose chief by-product is lactic acid.

The by-products of different kinds of bacteria growing in milk may be of such a nature that they will neutralize each other, as for example alkali may be neutralized by the formation of acid. Again the various groups of bacteria react upon each other in different ways, some being retarded by the associated organisms, while others are favored, a relation generally referred to under the expression "associative action."

To obtain any idea of the effect of this complex of bacteria in influencing the keeping quality of milk is a task that can not be accomplished with any degree of completeness, no matter what method may be called into service. It seems to the writers that some have been unduly optimistic in regard to the methods they have suggested for the purpose of measuring the quality of milk, and in some instances they have not had in mind the various relations, briefly mentioned above, that may exist between different bacteria and between their by-products in milk.

The desirability of a method that will enable the analyst, within the shortest possible time and with the minimum of labor and expense, to place a number of samples of milk in the same order, with reference to keeping quality, as he would place them by a detailed examination of the milks after they have been kept for twenty-four hours at 50° or 60°F. is beyond question. In other words the method should enable the analyst to take into account all those points which the milk consumer uses in judging whether the milk delivered to him is to be graded as good or poor. Among these will be the development of acid, the occurrence of abnormal odors and tastes in milk that is not acid to taste or smell, and the ease with which the milks curdle on heating. No one method can accomplish this. The problem is then to select from the various methods available the one that approximates the ideal most closely. The method must be applicable to both raw and pasteurized milk. The information it will supply may differ in value, this being dependent on the class of milk to which it is applied. These matters have been

considered more in detail in a previous article (3). To the methods there discussed should be added two that have been recently suggested (4) (5). The authors of the first of these papers state that it was hoped to find a way by which to measure the ability of the bacteria and enzymes present to bring about changes in milk which might make it unsuitable for the purpose for which intended, and which method would also take into account the associative action of bacteria in milk, something other methods fail to do. The method suggested by these investigators involves the inoculation of plain broth containing bromthymol blue, adjusted to a reaction of pH 7.0, with 0.1 cc. of milk to be examined and its subsequent incubation at 37°C. The rate of change of reaction in the broth is determined by reading its hydrogen ion concentration at definite times. It is at once evident that when a mixture of bacteria such as is present in milk, is seeded into a medium so different chemically and physically from milk as is plain nutrient broth the relations existing between the groups will not continue to be the same as they were in the milk at the time of the transfer or as they would have been in the milk at any moment in its future. The changes which the bacteria produce in broth are therefore not likely to be the same, or even to correlate in intensity with those which the same mixture of bacteria would cause in milk. The data presented in the paper are not especially convincing as to the value of the method in determining the keeping quality of milk.

The method outlined in the second paper involves the addition of an indicator, bromcresol purple, to the milk to be examined and noting the change in color after a period of incubation. The indicator in question reveals the gradual change in reaction of the milk as acid is formed by the bacteria. It is evident that the method measures primarily the acid-forming bacteria. Since these are the prime factors in determining the keeping quality of milk, it is quite possible that the results obtained may correlate well with the keeping quality. The authors suggest the use of four stages or degrees of acidity: the first is indicated by the first observable change from the

grayish-blue noted in normal milk with this indicator to a lighter color in any part of the milk; the fourth stage is one in which the color of the mixture of milk and indicator becomes a yellow, free from every trace of bluish or greenish tinge. The curdling of the milk usually occurs at this last stage. The four stages therefore include perfectly sweet milk at one extreme and milk that is curdled at the other. The commercial life of milk is practically at an end when the increase in acidity amounts to a few hundredths of one per cent. The bacterial content of such milk is so high that its keeping quality, when stored at 18 to 20°C, is measured usually by a few hours. The method is not one by which milks, still commercially valuable, i.e., those in which no appreciable acidity has developed, can be differentiated into a number of groups.

It may be asked whether either of these methods is as delicate, any more easily worked, or less expensive than some of the methods that have been in use for a considerable time. The methylene blue reduction test has been shown by one of the writers (3) to be an excellent way of determining the bacterial content of milk. From the data presented, the conclusion was drawn that it is as delicate as the plate culture method, and possibly even more delicate. The reduction test has all the advantages of the methods suggested in the papers to which reference has been made. By its use the milks can be placed in many groups, the only limit being the differences in the milks, and the frequency with which they are examined during the period of incubation. If the milks are examined every fifteen minutes during the first four hours and at thirty minute intervals thereafter during a second period of four hours, they could be placed in twenty-four groups. Less frequent examination would result in the milks being thrown into a smaller number of groups.

The reduction test apparently is influenced by all the bacteria that are growing in the milk at the temperature employed, since so far as is known all bacteria reduce methylene blue. It may be that even the living cells that are not reproducing exert an influence in this test. Some data have been obtained that would indicate this possibility. It is probable that the reduction test

reflects the interrelations of the various groups of bacteria on each other far more perfectly than does the method proposed by the Michigan workers, since the test is made in the milk in question and not by transferring a part of it to a medium which has different chemical and physical properties. The method proposed by the New York workers measures but one group of bacteria, the acid-formers. A method which measures the number of bacteria rather than their by-products should theoretically possess greater value in the examination of milk than a method which measures the by-products. From theoretical grounds the reduction test seems to approximate the ideal method more closely than does any other method.

It is not intended to convey the idea that the reduction test is influenced to the same extent by all bacteria. It will be shown in a later paper that all the bacteria in the milk are concerned in bringing about the reduction of the methylene blue. Furthermore, the application of the test itself is not possessed of the difficulties and uncertainties of the other two methods, namely, that of detecting delicate color changes either with the aid of a comparator or with the eye alone. Memory for delicate distinctions in shades of a color may easily change from day to day while it is doubtful that there would be any hesitation in deciding between color and absence of color as is the case in the end point for the reduction of methylene blue.

For the purpose of determining the relative value of the reduction test as compared with the use of bromcresol purple in milk, and bromthymol blue in broth inoculated with milk, several comparative tests were made. Pure cultures of typical milk organisms were used, and by means of definite dilutions an approximate idea was had of the relative number of organisms present in the samples tested. The methods used and the results obtained for the several trials were so similar that only a few representative comparisons will be given.

## EXPERIMENTAL

One hundred and forty cubic centimeter portions of fresh whole milk which had been heated to 100°C. for a short time were inoculated separately with 1 cc. of an eighteen hour old milk culture of the organism to be tested. Several strains of *B. coli* and of *B. lactis aerogenes*, and a few cultures isolated from milk, two of which are numbered 20.2 and 44.10 were used. From each 140 cc. of inoculated milk, nine 15 cc. portions were measured out. The first 15 cc. portion of each culture was inoculated with 1.5 cc. of a freshly curdled culture of *Bact. lactis acidii*, and of this dilution 10 cc. was transferred to another 15 cc., which method of dilution was continued until all nine of the 15 cc. portions of inoculated milk of the different series had received diminishing amounts of the lactic culture. A set of the different dilutions of the lactic culture alone was made in uninoculated heated milk. Each series therefore included eight samples of milk, decreasing in bacterial content from the first to the last. In the set in which *Bact. lactis acidii* alone was present the dilution was as follows:

Sample 1. 1 part of culture to	25 parts of milk
Sample 2. 1 part of culture to	62 parts of milk
Sample 3. 1 part of culture to	155 parts of milk
Sample 4. 1 part of culture to	390 parts of milk
Sample 5. 1 part of culture to	975 parts of milk
Sample 6. 1 part of culture to	2,437 parts of milk
Sample 7. 1 part of culture to	5,092 parts of milk
Sample 8. 1 part of culture to	12,730 parts of milk

In the sets in which *Bact. lactis acidii* was used with another organism, the inoculum of the latter was the same in each sample, while the lactic organism varied in number as described above. The result was a series of samples of milk of a gradually decreasing bacterial content. An accurate method of measuring the number of cells in the various samples in any set should place the samples in the order of their bacterial content.

Methylene blue reduction tests, bromthymol blue broth tests, and bromcresol purple milk tests were run on each dilution except the first. For the reduction test, 1 cc. of Merck's methylene

blue (1:20,000) was added to 10 cc. of the inoculated milk and reduction was carried out in a water bath at 37°C.

For the bromthymol blue test sterilized beef-peptone broth (pH of 7.0) to which had been added the indicator in the proportion of 4 drops of a 0.02 per cent solution to each 10 cc. of medium, was pipetted in 10 cc. portions into sterilized test tubes. One-tenth cubic centimeter of the milk to be tested was added direct to the broth and the tubes were incubated in a 37°C. water bath. Color controls were made by adding an equal amount of heated uninoculated milk to 10 cc. of the bromthymol blue broth, and changes in reaction were determined at the end of one and two hours incubation by comparing the inoculated tubes with the uninoculated for any variation in color. What is reported in table 1 as a slight change was not more than 0.1 in the pH scale of hydrogen ion values.

The bromcresol purple test was made by adding 5 cc. of the milk dilutions of the cultures to sterilized tubes containing 0.05 cc. of the indicator (1 part in 1000 parts of water). These were also incubated at 37°C. and readings were made at the end of one, two and twenty-four hour periods for any variation in color from that of the control.

In table 1 the changes in bromthymol blue and bromcresol purple are recorded only after the two hour incubation, because most of the cultures in practically all of the dilutions showed no change at the end of one hour. These two indicators were not influenced by the number of bacteria except where this was very marked, as in the first dilutions, whereas the reduction time of methylene blue in all cases tended to vary directly according to the number of organisms present. When growth took place throughout the whole of a series of dilutions in broth, the change in reaction, as shown by the color of the indicator, was the same for all except usually the first dilution. No such gradation of milks, based on the number of bacteria present, as was obtained with methylene blue, ever resulted with the two indicators bromthymol blue and bromcresol purple. The bromcresol purple has shown in two hours time even less gradation than the bromthymol blue, and when incubation was continued for twenty-

TABLE 1

*Comparison of the methylene blue reduction test, the bromthymol blue test, and the bromcresol purple test on milks inoculated with varying amounts of pure cultures*

	SAMPLE							
	1	2	3	4	5	6	7	8
<b>Bact. lactis acid</b>								
Methylene blue reduction time in minutes	6	30	57	117	162	222	283	358
Bromthymol blue after 2 hours at 37°C.	++	+	+	+	+	+	+	+
Bromcresol purple after 2 hours at 37°C.	++	-	-	-	-	-	-	-
<b>Bact. lactis acid and culture 20.2</b>								
Methylene blue reduction time in minutes	7	29	52	61	71	81	90	105
Bromthymol blue after 2 hours at 37°C.	+++	+++	+++	+++	+++	+++	+++	+++
Bromcresol purple after 2 hours at 37°C.	+	-	-	-	-	-	-	-
<b>Bact. lactis acid and culture 44.10</b>								
Methylene blue reduction time in minutes	5	24	52	71	85	102	103	118
Bromthymol blue after 2 hours at 37°C.	+++	++	+	+	+	+	+	+
Bromcresol purple after 2 hours at 37°C.	++	+	-	-	-	-	-	-
<b>Bact. lactis acid and B. coli</b>								
Methylene blue reduction time in minutes	7	21	52	71	93	115	131	190
Bromthymol blue after 2 hours at 37°C.	+	+	+	+	+	+	+	+
Bromcresol purple after 2 hours at 37°C.	+	-	-	-	-	-	-	-
<b>Bact. lactis acid and B. aerogenes A<sub>2</sub></b>								
Methylene blue reduction time in minutes	7	29	63	89	96	110	131	147
Bromthymol blue after 2 hours at 37°C.	++	+	+	+	+	+	+	+
Bromcresol purple after 2 hours at 37°C.	+	-	-	-	-	-	-	-

TABLE 1—Continued

	SAMPLE							
	1	2	3	4	5	6	7	8
<b>Bact. lactis acidi and B. aerogenes B<sub>2</sub></b>								
Methylene blue reduction time in minutes	6	29	58	82	100	118	142	156
Bromthymol blue after 2 hours at 37°C.	+++	+++	+++	+++	+++	+++	+++	+++
Bromcresol purple after 2 hours at 37°C.	—	—	—	—	—	—	—	—

— No change; + very slight; ++ slight change; +++ decided change.

four hours the maximum color change of the indicator was reached in all the different dilutions.

The conclusion to which one is forced is that neither of the methods is as delicate nor as accurate a measure of the bacterial content of milk as is the reduction test. They did not enable the analyst to place the varying dilutions in their proper order in respect to the number of bacteria while the reduction test failed in this in no instance. The few data presented are typical of all collected in making over two hundred such trials.

In order to compare the three tests on milks of unknown bacterial content, market milks which were anywhere from four to twenty-four hours old were used. In making the bromthymol blue test, the inoculum for the broth was 1 cc. of a 1 to 10 dilution of the milk in water instead of 0.1 cc. of milk as previously used. For the bromcresol purple test 5 cc. of milk and 0.05 cc. of indicator were used. The results are reported in tables 2 and 3.

With these milks the results were similar to those obtained with pure cultures in milk; neither of the indicators was so sensitive to the biological differences in the milks as was the methylene blue. The bromthymol blue test when applied to milks of widely different keeping quality, showed in a general way rough agreement with the methylene blue, but it would not divide the milks into as many classes as did the latter. The tubes of broth inoculated with milks which were at the curdling point did not show a change in reaction in an hour while the



reduction time for such milks is very short, a minute or less. In the case of milks that had a reduction time of two to thirty minutes, which means that such milks have passed the point where they are marketable, from two to five hours were required

TABLE 2

*Methylene blue reduction time of milks compared with time for first change in bromthymol blue broth*

SAMPLE OF MILK	METHYLENE BLUE REDUCTION TIME	BROMTHYMOL BLUE COLOR CHANGE
	minutes	minutes
1	1	75
2	2	285
3	3	135
4	3	300
5	4	300
6	8	180
7	11	151
8	21	270
9	27	300
10	30	300
11	36	285
12	58	300
13	200	300
14	240	420
15	280	300
16	295	300
17	360	480
18	420	No change in 480
19	450	540
20	480	600, change doubtful
21	480	600, change doubtful
22	510	No change in 600
23	540	600
24	540	540
25	570	No change in 650
26	Slight reduction in 570	600
27	Slight reduction in 570	No change in 600
28	Slight reduction in 570	600, change doubtful
29	No reduction in 570	540
30	No reduction in 570	540

before a change was noted in the color of the bromthymol blue broth. Milks with a reduction time of eight to ten hours required only eight to ten hours for a change in color of bromthymol blue to become evident.

The variations in the color itself of different milks cause variations in the color of the bromthymol blue broth to such an extent that considerable error may occur unless a control tube is made from each milk tested with which the sample that has been incubated can be compared.

TABLE 3

*Methylene blue reduction time of milks compared with their color reaction with bromcresol purple*

SAMPLE OF MILK	METHYLENE BLUE REDUCTION TIME	BROM-CRESOL PURPLE COLOR CHANGE
	minutes	
1	1	Yellow
2	1	Yellow
3	9	Lighter than control
4	23	Lighter than control
5	46	Same as control
6	84	Greenish tinge
7	134	Greenish tinge
8	136	Same as control
9	189	Darker than control
10	189	Lighter than control
11	210	Darker than control
12	260	Lighter than control
13	280	Darker than control
14	300	Greenish
15	314	Lighter than control
16	314	Greenish tinge
17	314	Lighter than control
18	344	Same as control
19	350	Same as control
20	No change in 8 hours	Slightly lighter than control
21	No change in 8 hours	Slightly lighter than control
22	No change in 8 hours	Slightly lighter than control

The failure of the bromthymol blue broth to differentiate between milks to the degree that the methylene blue does might be attributed to several things. Diluting milks of a varying bacterial content might tend to overcome the variations. Instances are not uncommon in plate cultures where a dilution falls far short of having the theoretical number of organisms when compared with the preceding dilution. As suggested be-

fore, rearrangement of the relation among the different groups of organisms present in a milk both by its dilution and by its inoculation into a different medium, and subsequent adjustment to a new environment would also tend to overcome the original variations in the milks.

The bromcresol purple test when used with milks ranging from a few to twenty-four hours old brought out marked differences, but conclusions as to their keeping quality based on these differences could not be relied upon because of the numerous factors, such as color of the milk, fat content, and total solids, which may influence the color of this indicator. Examples were found of milks which, according to their methylene blue reduction time, would be at opposite ends when classified in respect to their keeping quality, gave with bromcresol purple the same color. The light color with the two milks reducing in nine and twenty-three minutes, table 3, was no doubt due to growth; but the same color in a milk with a reduction time of five hours was of course due to some factor other than growth. It is also quite probable that acid in amounts sufficient to be detected by bromcresol purple was formed in a milk with a reduction time of forty-six minutes and could have been detected by this indicator provided a control tube of the same milk in which no growth had taken place had been available. Thus it is evident that large numbers of bacteria may grow in some milks without changing the color of the indicator to the degree that an acid reaction can be detected, and it is even possible that in some milks the hydrogen ion concentration might not be changed though considerable growth had taken place. Especially would this be the case in a milk high in total solids and basic salts where any acid that was formed would be taken up by these constituents. Furthermore, a neutral reaction might be maintained in a milk for a considerable length of time because of the balance produced by the growth of acid- and alkali-producing organisms. Such a milk would have a high bacterial content before the acid formers would affect the color of the indicator. If a milk were low in total solids, it would give a darker blue with bromcresol purple than a normal milk and in order to bring about the same

color in the acid direction in the two milks, it would require in the case of the abnormal milk either the growth of a greater number of acid-producing organisms or a longer period of growth than would be required in the milk having a normal color at the start.

#### CONCLUSION

The results herein reported show that the methylene blue reduction test is a more accurate measure of the number of bacteria in milk than is either the bromthymol blue or the bromcresol purple test. These two tests depend on the measurement of acid, one of the by-products of the growth of bacteria, whereas the reduction of methylene blue depends on the number of living cells.

The reduction test involves no more labor or time than do the other tests and is more easily carried out by untrained workers. And these in using either of the indicator tests would undoubtedly arrive at different conclusions in judging the quality of a series of milks because of the possible errors and difficulties in interpreting the tests. Interpretation of the methylene blue test might in a few instances present difficulties, but these have been met with so seldom that they might be considered negligible. It is to be expected that the methylene blue test in its application will reveal some discrepancies, but these are not, in the experience of the writers, as obvious as those in the bromthymol blue and the bromcresol purple tests.

In a subsequent paper it will be shown that different kinds of bacteria do not have the same reducing power; and that the lactic acid bacteria which are of the greatest importance in the spoiling of milk are also most active in the reduction of methylene blue, while other common milk bacteria such as those of the *B. coli-aerogenes* group which are of secondary importance in the spoiling of milk, are likewise less active in the reduction of methylene blue. It may well be, therefore, that the reduction test will prove to be an excellent measure of the keeping quality of milk.

## SUMMARY

In a comparison of the three tests, methylene blue reduction, bromthymol blue, and bromocresol purple for the determination of the keeping quality of milk, it is shown that the methylene blue reduction test is the preferable because of its greater sensitiveness to biological differences in milks; because it measures the number of bacteria rather than the by-products of their growth; and because of its simplicity and practicable advantages.

## REFERENCES

- (1) HARDING, H. A., BREED, R. S., STOCKING, W. A., AND HASTINGS, E. G.: What is meant by "quality" in milk. *Journal Dairy Science*, i, 199-217. 1917.
- (2) HARDING, H. A. AND PRUCHA, M. J.: Suggestions regarding the control of municipal milk supplies. *Journal Dairy Science*, iii, 107-121. 1920.
- (3) HASTINGS, E. G.: The comparative value of quantitative and qualitative bacteriological methods as applied to milk, with especial consideration of the methylene blue reduction test. *Journal Dairy Science*, ii, 293-311. 1919.
- (4) COOLEGE, L. H., AND WYANT, R. W.: The keeping quality of milk as judged by the colorimetric hydrogen ion determination. *Journal Dairy Science*, iii, 156-166. 1920.
- (5) BAKER, J. C., AND VAN SLYKE, L. L.: Tech. Bul. No. 72, New York Agr. Expt. Station (Geneva). 1919.

# THE DIGESTIBILITY OF SORGHUM MILL REFUSE<sup>1</sup>

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The sorghum mill was at one time a common institution in many sections but in prewar days the number in operation had become greatly reduced. The sugar shortage during the last few years has again led to the operation of a large number of them. The amount of sorghum grown for syrup is not great in this section but yet questions regarding the feeding value of the sorghum mill refuse are frequent.

No information is available on this point so the present work was undertaken to determine the value of this product as a feed for livestock.

## EXPERIMENTAL METHODS

The sorghum refuse was obtained from a local mill and consisted of the stalks remaining after the extraction of the juice. It was generally about two days old when used and was in quite fair condition. It was fed uncut.

The length of the feeding period was 12 days and during the last five days the digestion trial was conducted according to the methods generally recognized. Salt was given the animals at free will and they were watered twice daily.

The work was started on October 5, 1919, and where necessary the information given concerning the animals used is calculated to that date. Cow 91 was a purebred Ayrshire and cow 262 was a Guernsey-Ayrshire cross. Both animals had been used for a considerable time in other feeding and digestion trials.

The weights of the feed consumed and the faeces produced were determined daily. The faeces samples were dried and composited and these with the samples of air dried feed were the materials on which the proximate constituents were determined

<sup>1</sup> The coöperation of W. G. Gaessler in undertaking the analytical work for this project deserves acknowledgment here.

TABLE 1  
*Animals used*

	cow 91	cow 262
Age.....	12 y., 1 m., 3 d.	5 y., 2 m., 18 d.
Days bred.....	0	158
Weight, pounds.....	1000	1080
Previous lactations.....	6	1

TABLE 2  
*Feed consumed and faeces produced*

	cow 91	cow 262	AVERAGE
	pounds	pounds	pounds
Feed.....	78.0	92.2	85.1
Faeces.....	97.5	105.7	101.6

TABLE 3  
*Analyses of feed and faeces*

	SORGHEUM MILK REFUSE	FAECES		
		Cow 91	Cow 262	Average
	per cent	per cent	per cent	per cent
Moisture.....	73.12	85.27	84.74	85.00
Dry matter.....	26.88	14.73	15.26	15.00
Crude protein.....	0.62	1.09	1.21	1.15
Nitrogen-free-extract.....	12.98	7.01	7.19	7.11
Crude fiber.....	11.48	4.72	5.42	5.08
Crude fat.....	0.46	0.22	0.21	0.21
Ash.....	1.34	1.69	1.23	1.45

TABLE 4  
*Nutrients consumed, defecated and digested*

	CONSUMED			DEFECATED			DIGESTED		
	Cow 91	Cow 262	Average	Cow 91	Cow 262	Average	Cow 91	Cow 262	Average
	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds
Dry matter.....	20.97	24.78	22.88	14.36	16.13	15.25	6.61	8.65	7.63
Crude protein.....	0.48	0.57	0.53	1.06	1.28	1.17	-0.58	-0.71	-0.65
Nitrogen-free-extract.....	10.13	11.97	11.05	6.84	7.60	7.22	3.29	4.37	3.83
Crude fiber.....	8.95	10.58	9.77	4.60	5.73	5.17	4.35	4.85	4.60
Crude fat.....	0.36	0.42	0.39	0.21	0.22	0.22	0.15	0.20	0.18

TABLE 5  
*Digestion coefficients and digestible nutrients*

	DIGESTION COEFFICIENTS			DIGESTIBLE NUTRIENTS
	Cow 91	Cow 262	Average	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Dry matter.....	31.5	34.9	33.4	9.0
Crude protein.....	0.0	0.0	0.0	0.0
Nitrogen-free-extract..	32.5	36.5	34.7	4.5
Crude fiber.....	48.6	45.8	47.1	5.4
Crude fat.....	41.7	47.6	44.9	0.2

by the recognized methods. All analyses were then computed back to the moist basis and only the analyses of the original undried materials are presented here.

#### DISCUSSION OF RESULTS

The average coefficients of digestibility for the sorghum mill refuse were low, that of the dry matter being 33.4 per cent. The crude protein was apparently undigested due to the fact that the amount of protein present was not sufficient to maintain the nitrogen balance in the animals and the presence of metabolic products caused the elimination of more crude protein in the faeces than there was in the feed.

The amounts of digestible nutrients present are small, the total content being only 10.4 per cent. When the net energy value of the sorghum mill refuse is calculated by the method of Armsby<sup>2</sup> it is found to be only 6.5 therms or a lower net energy value than has been reported for any other feed.

#### SUMMARY

The digestibilities of the nutrients in sorghum mill refuse are low and the net energy value is exceptionally low so there appears to be little opportunity for the extensive use of this waste produce as a feed.

<sup>2</sup> H. P. Armsby and J. A. Fries. Net energy values for ruminants. 1916, Bul. Pa. Agr. Exp. Sta. 142.



# THE DIGESTIBILITY OF CORN CANNERY REFUSE<sup>1</sup>

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The canning of sweet corn has now reached enormous proportions and huge quantities of refuse have to be disposed of in connection with these activities. A considerable quantity of this material is fed but a great proportion of it has in the past been allowed to go to waste. In recent years, however, the interest in this as a feed for farm livestock has been increasing and the work outlined here was undertaken with the object of obtaining some information regarding the value of the refuse from sweet corn canning factories. No work has as yet been reported on this problem and the material herein presented is merely indicative of the need for seriously considering the utilization of this waste product.

## EXPERIMENTAL METHODS

The corn cannery refuse used was obtained from a local factory and was generally two days old when fed. It was partially fermented and not in first class condition for feeding purposes as there was no means of handling it at the factory except by dumping. It consisted mainly of the sweet corn husks with only small amounts of spoiled ears and cobs as these generally went into separate piles. The material, when fed, had a bad odor but was consumed in fair quantities by the animals used in the experiment.

The feeding period was of twelve days duration and during the last 5 days the digestion trial was conducted according to the recognized methods. Salt was given at free will and the animals were watered twice daily.

The work was started on September 15, 1919, and where necessary the data given concerning the cows is calculated to that date. Cow 91 was a purebred Ayrshire while cow 262 was a

<sup>1</sup> The cooperation of W. G. Gaessler in undertaking the analytical work for this project deserves acknowledgment here.

Guernsey-Ayrshire cross and both had been used for a considerable time in feeding trials previous to being used for this work.

The weights of feed consumed and faeces produced were obtained daily and the faeces samples were dried and composited.

TABLE 1  
*Animals used*

	cow 91	cow 262
Age.....	12 y., 0 m., 13 d.	5 y., 1 m., 28 d.
Days bred....	0	138
Weight, pounds.....	1005	1090
Previous lactations....	6	1

TABLE 2  
*Feed consumed and faeces produced*

	cow 91	cow 262	Average
	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
Feed.....	219 75	249 50	234 63
Faeces.....	182 75	158 00	170 38

TABLE 3  
*Analyses of feed and faeces*

	CORN CANNERY REFUSE	FAECES		
		Cow 91	Cow 262	Average
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Moisture.....	83 28	86 57	81 73	84 33
Dry matter.....	16 72	13 43	18 27	15 67
Crude protein.....	1 32	1 53	1 85	1 68
Nitrogen-free-extract.....	9 17	6 59	8 74	7 59
Crude fiber.....	5.02	4 38	6 20	5.22
Crude fat.....	0 34	0 16	0 40	0 27
Ash.....	0 87	0 77	1 08	0 91

The proximate constituents of these samples and the air-dry sample of feed were determined by the recognized methods. All analyses were calculated back to the moist basis and are reported here on the basis of the original materials.

## DISCUSSION OF RESULTS

The average digestion coefficients for the corn cannery refuse were quite low, that for the dry matter being 31.9 per cent. Of the individual constituents, those occurring in the smallest amounts, namely fat and protein, showed the greatest variations. The average coefficient of digestibility for the fat was 42.5 per cent and that for the crude protein was only 7.7 per cent. In

TABLE 4  
*Nutrients consumed, defecated and digested*

	CONSUMED			DEFECATED			DIGESTED		
	Cow 91	Cow 262	Average	Cow 91	Cow 262	Average	Cow 91	Cow 262	Average
	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds	pounds
Dry matter.....	36.74	41.72	39.23	24.54	28.87	26.71	12.20	12.85	12.53
Crude protein....	2.90	3.29	3.10	2.80	2.92	2.86	0.10	0.37	0.24
Nitrogen-free-extract.....	20.15	22.88	21.52	12.04	13.81	12.93	8.11	9.07	8.59
Crude fiber.....	11.03	12.53	11.78	8.00	9.80	8.90	3.03	2.73	2.88
Crude fat.....	0.75	0.85	0.80	0.29	0.63	0.46	0.46	0.22	0.34

TABLE 5  
*Digestion coefficients*

	cow 91	cow 262	Average
	per cent	per cent	per cent
Dry matter.....	33.2	30.8	31.9
Crude protein.....	3.5	11.2	7.7
Nitrogen-free-extract.....	40.2	39.6	39.9
Crude fiber.....	27.5	21.8	24.4
Crude fat.....	61.3	25.9	42.5

addition the apparent abilities of the animals so far as digestion was concerned varied greatly in the case of the fat and the protein, cow 91 having a digestion coefficient of 61.3 per cent for fat and 3.5 per cent for crude protein while the corresponding figures for cow 262 were 25.9 per cent and 11.2 per cent. The animals showed quite uniform ability so far as the digestion of the two chief constituents of the dry matter, nitrogen-free extract and crude fiber, were concerned.

A better appreciation of these results can perhaps be obtained if a comparison be made between the corn cannery refuse and corn silage. The comparison is made with corn silage rather than with corn stover silage as no digestion trials have been reported with stover silage.

When the comparison between the corn cannery refuse and corn silage is made it is found that the refuse contains about 10

TABLE 6  
*A comparison of corn silage and corn cannery refuse\**

	COMPOSITION		DIGESTION COEFFICIENTS		DIGESTIBLE NUTRIENTS		DIGESTIBLE NUTRIENTS IN 1 TON		
	Corn silage	Cannery refuse	Corn silage	Cannery refuse	Corn silage	Cannery refuse	Corn silage	Cannery refuse	Cannery refuse and 5 bushels corn
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>			
Moisture.....	73.7	83.3							
Dry matter.....	26.3	16.7	66	32	17.4	5.3	348	106	
Crude protein.....	2.1	1.3	51	8	1.1	0.1	22	2	23
Nitrogen-free-extract.....	15.4	9.2	71	40	10.9	3.7	218	74	264
Crude fiber.....	6.3	5.0	65	24	4.1	1.2	82	24	27
Crude fat.....	0.8	0.3	82	43	0.7	0.1	14	2	15
Ash.....	1.7	0.9							
Carbohydrate.....					15.0	4.9	300	98	291
Carbohydrate equivalent.....					16.6	5.1	332	102	325
Total.....					17.7	5.2	354	104	348
Nutritive ratio.....							1:15	1:51	1:14

\* The data on corn silage are from Feeds and Feeding, W. A. Henry & F. B. Morrison, 17th edition, 1917.

per cent more moisture than does the silage and so is correspondingly low in the valuable nutrients, this being especially true in the case of the fat. The coefficients of digestibility for the corn cannery refuse are much lower than those for the silage and the most marked decrease is found in the case of the apparent digestibility of the crude protein. This was probably due to the fact

that the relatively small amount of protein in the feed allowed the results to be masked by the metabolic products in the faeces.

The content of digestible nutrients differs widely for the two feeds, being 17.7 per cent of total digestible nutrients in the case of the corn silage and 5.1 per cent in the case of the corn cannery refuse. The relative difference is most marked in the case of the crude protein and fat.

Another method of comparing the two feeds is to study the amount of digestible nutrients in 1 ton of each. A ton of corn silage contains 22 pounds of digestible crude protein and 332 pounds of digestible carbohydrate equivalent or 354 pounds of total digestible nutrients, while 1 ton of the corn cannery refuse studied contained only 2 pounds of digestible crude protein and 102 pounds of digestible carbohydrate equivalent or 104 pounds of total digestible nutrients. In other words, 1 ton of corn silage contains about as much digestible nutrients as  $3\frac{1}{2}$  tons of the cannery refuse. The nutritive ratio of corn silage is 1:15 while that of the refuse is 1:51.

The relative feeding values of the two feeds can not be properly appreciated from these figures but if 5 bushels of corn, the average amount in 1 ton of silage, be added to 1 ton of the refuse it is found that this combination will contain 23 pounds of digestible crude protein and 325 pounds of digestible carbohydrate equivalent or 348 pounds of total digestible nutrients. This means that 1 ton of corn cannery refuse and 5 bushels of corn will have a feeding value approximately equal to that of 1 ton of good corn silage.

#### SUMMARY

Large amounts of corn cannery refuse are allowed to waste but if proper methods of storage and handling were instituted a considerable amount of valuable feed could be saved as 1 ton of corn cannery refuse and 5 bushels of corn are apparently of about the same feeding value as 1 ton of good corn silage.

# A BACTERIOLOGICAL AND BIOCHEMICAL STUDY OF EXPERIMENTAL BUTTERS<sup>1</sup>

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Originally, this investigation was intended as a study of the effects of cream ripening and of pasteurization upon the keeping qualities of butter. Owing to the fact, however, that considerable time has elapsed since the work was done and in this time some of the methods and ideas of manufacture have undergone some changes, it is thought best to present the results from the point of view expressed in the title.<sup>2</sup> The reasons for this decision will become clearer as the work is presented. It is hoped that the data to be presented will be of value to bacteriologists and chemists studying this problem, if not to practical butter makers.

While the purpose of this investigation was to study the effect of ripening and pasteurization on the keeping quality of the butter, the effects of other treatments of the butter were also to be observed. These included, (a) washing of the butter with lactic acid, (b) the addition of casein, (c) the addition of fishy butter, and (d) the addition of boric acid.

A single vat of cream was divided into two equal portions, one of which was pasteurized and the other unpasteurized. Each of these portions was further subdivided into two parts, one part being churned without further treatment and the other ripened by the addition of starter. Each of the four divisions of cream was then churned separately. The butter from each churning was then divided into five portions which were subjected to the four further treatments mentioned above, with one of

<sup>1</sup> Published by permission of the Director of the Michigan Experiment Station, East Lansing, Mich.

<sup>2</sup> The work presented here was done during 1909 and 1910 by Chas. W. Brown and Lulu M. Smith but was not published. Mr. Ruehle is not responsible for the plan of the work nor for the gathering of the data, but is largely responsible for the presentation of the results in written form.

the portions acting as an untreated control. The twenty different butters (about 60 pounds each) were packed in 30 pound tubs and shipped without delay to a cold storage plant where they remained for the duration of the experiment at about 0°F. At intervals of 9, 48, 164, 275, and 426 days the butters were scored and sampled for bacteriological and chemical determinations.

The following scheme indicates the divisions of the cream and the treatments of the butters:

		<i>Sample</i>	<i>Treatments</i>
Cream 28.18 per cent fat 53° acidity	Raw	Part 1, churned immedi- ately, 53° acid	1 and 11 Control
			2 and 12 Acidified H <sub>2</sub> O in 2d wash- ing
			3 and 13 Casein added
			4 and 14 Fishy butter added
			5 and 15 Boric acid added
		Part 3, to 60°	21 and 31 Control
			22 and 32 Acidified H <sub>2</sub> O in 2d wash- ing
			23 and 33 Casein added
			24 and 34 Fishy butter added
			25 and 35 Boric acid added
	Pasteurized at 160°C.	Part 2, churned immedi- ately	6 and 16 Control
			7 and 17 Acidified H <sub>2</sub> O in 2d wash- ing
			8 and 18 Casein added
			9 and 19 Fishy butter added
			10 and 20 Boric acid added
		Part 4, to 64°	26 and 36 Control
			27 and 37 Acidified H <sub>2</sub> O in 2d wash- ing
			28 and 38 Casein added
			29 and 39 Fishy butter added
			30 and 40 Boric acid added

A more detailed description of the methods of manufacture of the butters and the methods of bacteriological and chemical investigations follows.

## SECURING THE CREAM

The cream was secured on a prearranged date (October 8, 1909) from three near-by creameries; from the first was received 1365 pounds of 28.91 per cent butterfat, from the second 1471 pounds of 26.93 per cent butterfat and from the third 1118 pounds of 28.93 per cent butterfat—a total of 3954 pounds of 28.18 per cent butterfat. The cream from the first creamery, all gathered cream, arrived in good condition; that from the second, all gathered cream, was sour when it arrived; that from the third, about half gathered and half whole milk cream, arrived in good condition. The three lots were placed in a cool room until the following morning then they were turned into a large vat and stirred to make a homogeneous mixture.

## TREATMENT OF CREAM

The mixed cream in the vat contained 28.18 per cent butterfat and had an acidity of 53°. A slight metallic flavor, possibly derived from some of the cans used in shipping, was present. Part I was churned at once. Part III received an addition of 5 per cent starter (69° acid) and was held at 60°F. for about five hours then cooled to 50°F. and held over night. The ripened cream had an acidity of 60°. Part II was chilled immediately after it had been pasteurized at 160° in a Farrington pasteurizer and was churned about an hour later. No starter was added. Part IV received an addition of 5 per cent starter and was held for five hours at 60°F then cooled to 50°F. and held over night at that temperature. At time of churning the cream had an acidity of 64°.

## CHURNING AND STORAGE

The churning was done in the College Dairy under the supervision of L. B. Liverence, in charge of dairy manufacture. Butter from each churning was treated as follows:

*Butter washed with lactic acid.* After the first washing, the butter (60 pounds) was placed in a small churn and 15 pounds of a 1 per cent commercial lactic acid solution was added. The



churn was given five revolutions and then the acidified water was allowed to drain away. Dry salt was added and the butter was worked sufficiently to incorporate it, after which the butter was packed into two thirty pound tubs.

*Butter with added casein.* To 60 pounds of salted butter in a small churn was added 90 grams powdered casein, prepared from sweet separated milk by precipitating with lactic acid and washing successively with water alcohol, absolute alcohol, and ether. The butter was worked to incorporate the casein—the water being allowed to drain away—and was packed into two 30 pound tubs.

*Butter with boric acid added.* One hundred and twenty grams of powdered boric acid was worked into 60 pounds of salted butter; the excess water was allowed to drain away and the butter was packed into two 30 pound tubs.

*Butter with fishy butter added.* One pound of fishy butter which was obtained from a tub of butter scored as having a pronounced fishy flavor by the Michigan Dairy and Food Department at a judging contest in Grand Rapids, was worked into 60 pounds of salted butter. The butter was placed in two 30 pound tubs. The fishy butter used in this work was analyzed bacteriologically and was found to contain 2,300,000 bacteria per gram. The flora isolated consisted of non-liquefying yeast, *M. lactis rosaceus* and a number of unidentified species.

*Unmodified butter.* The remainder of the churning was also tubbed; two 30 pound tubs being used as controls.

*Storage of butter.* The tubs were numbered and expressed immediately to the Booth Cold Storage at Detroit where a temperature was maintained at 0°F. Twenty-four hours before each scoring the tubs were placed in a comparatively warm ante-room to the cold storage, being returned to the storage room as soon as scored.

#### SCORING AND SAMPLING

When the butter was 9, 48, 164, 276 and 426 days old it was judged independently by F. O. Foster, G. A. Gilbert and L. B. Liverence. The average score with the descriptive terms used

are recorded in table 1, and will be discussed on page 380. At the time of each scoring two sets of samples were taken from one of each pair of tubs. For the first set about 50 grams were taken with a sterile trier, placed in sterile morphine bottles and used the following day for bacteriological analyses and for the determination of acidity, moisture and salt. For the second set of samples about 1 pound was taken with a knife, placed in mason jars—no surface butter was included—and was used the second day following for chemical analyses. All samples were kept cold from the time of collecting to the time of analyzing.

#### PLATING AND ANALYTICAL METHODS

The bottle containing the sample for bacteriological analysis was placed in a water bath at 35°C. until the butter softened. The cork was removed and the butter stirred with a sterile glass rod, then 11.5 cc. (10 grams) of butter were introduced by means of a sterile pipette into 90 cc. of warm (35° to 40°C.) sterile physiological salt solution; from this the higher dilutions were made. Nine plates were made from each sample; one set of three (three different dilutions) was poured with litmus lactose agar (standard meat infusion agar with acidity of 10°F.S. and containing 1 per cent lactose and 0.025 per cent azolitmin) and two sets were poured with lactose salt agar (same as above without azolitmin and containing 10 per cent sodium chlorid). The litmus lactose agar plates were incubated at 20° to 22°C. for five to seven days then isolations and counts were made. One set of lactose salt agar was incubated at 20° to 22°C. for ten to fourteen days and the other set at 5° to 10°C. for about twenty days. At this time isolations and counts were made.

The methods for the determination of acidity, moisture, salt, total nitrogen, nitrogen not precipitated by copper sulphate, and acidity of butter leachings are the same as those employed in a previous study (Technical Bul. 2, Mich. Agr. Sta., pp. 13–15) with the exception that the acidity of butter is recorded as cubic centimeters of N/10 acid per 100 grams butter.

*Lactose* was determined according to the method described by Benedict (1) (1907). One hundred cubic centimeters of the

filtrate from the copper sulphate precipitation is placed in a 100 to 110 cc. sugar flask, a few drops of phenolphthalein is added and N/1 sodium hydroxid is added drop by drop until the copper is precipitated (the phenolphthalein indicates when enough alkali is added). The volume is made up to 110 cc. and filtered. Ten cubic centimeters of Benedict's reducing solution are placed in a small Erlenmeyer flask and heated to boiling. The lactose solution is run in from a burette, the number of cubic centimeters required to reduce the copper solution is read and the amount of lactose is computed and recorded as percentage of butter.

### RESULTS

The results of the scorings on flavor and keeping quality and of the bacteriological studies so far as the seedings made on lactose agar incubated at 19°C. are concerned, are presented in table 1. The results of the other platings will be discussed but not presented in full, owing to lack of space.

Table 1 needs a word of explanation. The scorings are presented under headings as "sample" and "duplicate sample." These are two tubs of butter in each case, as numbers 1 and 11, 2 and 12, etc. The bacterial and chemical determinations, however, were only made on one of the tubs and represent the butters listed under the heading "sample." The heading "flora isolated" should not be misinterpreted as meaning that the organisms listed were the only ones isolated. They represent the only ones which survived long enough to be wholly or partially identified. The term "lactic" in the table refers to organisms which produced a small acid colony on the litmus lactose agar plates. When some of these were transplanted to litmus milk they produced the typical lactic acid curd accompanied by a reduction of the litmus they all are, therefore, in all probability true members of the species, *Bacterium lactis acidii* or *Streptococcus lacticus*.

Inspection of the results in table 1 fails to reveal any very definite relationship between score and the number or kinds of bacteria or between the development of the particular off-flavors and the bacterial flora present.

Most of the raw cream butters had developed the old cream flavor at the end of nine days when the first scorings were made while the pasteurized cream butters had developed a metallic flavor. The old cream flavor usually was followed with increasing age by fishy flavor while the metallic flavor was not. This suggests that pasteurization may have had some effect in preventing this particular flavor, which in turn supports the idea suggested by Supplee (2) that a biological agent is concerned in the development of this flavor.

The development of the metallic flavor on the other hand seems to have been associated in these butters more with the pasteurized cream butters than with those made from raw cream. Possibly this was due to the use of a pasteurizer the surfaces of which had copper exposed and the combination of acid and high heat favored the formation of copper salts. In this case, however, one would expect the development, later, of tallowy flavor as shown by the results of Hunziker and Hosman (3) and those of Palmer and Combs (4). This, however, is not the case. The tallowy flavor developed much more frequently in the raw than in the pasteurized samples. The other flavors reported, occurred so infrequently that no definite conclusions can be drawn from them. It should be noted, however, that the acrid flavor developed much more frequently in the well ripened butters and in these latter more frequently in the pasteurized samples than in the raw samples. No reason has been assigned for this peculiarity.

*Bacteriological findings.* The most striking results of the bacteriological work were the following: (1) The relatively high counts of the butters when over a year old. There is a sharp falling off in numbers during the first nine days, after which the numbers gradually decline but leave higher counts than are usually reported for old butters.

(2) The relatively prolonged vitality of the lactic flora. In pure cultures in milk, which is usually considered the best medium for the growth of this species, the lactics usually die off within a week. In these butters, however, we find them persisting for as long as 275 days (and in one case for 426 days—sample 22).

TABLE 1

AGE	SCORE		CRITICISMS OF JUDGES		COUNT OF COLONIES PER GRAM OF SAMPLE	FLORA ISOLATED
	Sample	Duplicate	Sample	Duplicate sample		
Samples 1 and 11 (duplicate). Raw cream (53°) churned at once [Control]						
days Fresh						
9	92	91	Old cream, unclean	Old cream	20,500,000	Lactic, non-liquefying yeast, *Oidium
48	88½	88½	Old	Old cream	2,300,000	Lactic, *Oidium
164	88	88	Old cream, fishy, tallowy	Old cream	2,800,000	Lactic, *liquefying yeast
275	88	87½	Old cream, fishy, tallowy	Old cream, strong, dish-rag	2,100,000	Lactic. *B. subtilis, non-liquefying yeast
426	86½	85½	Fishy, tallowy	Unclean, metallic	1,750,000	Lactic, Bact. lactis syn-xanthum, M. lactis roseaceus
					1,900,000	*Liquefying yeast
Samples 2 and 12 (duplicate). Raw cream (53°) churned at once, butter washed with lactic acid						
9	91	93½	Old cream	Old cream	1,550,000	Lactic, *liquefying yeast, *Oidium
48	88	88	Old, unclean	Old cream, unclean	2,000,000	Lactic, *B. subtilis, *B. lactis tetragenus, *Oidium
164	89	85	Old cream	Fishy, rancid, moldy, unclean	1,800,000	Lactic, *B. subtilis, *Liquefying yeast, *Oidium
275	88	87½	Old cream, metallic	Old cream, stale, metallic	910,000	Lactic, *M. lactis aureus, *liquefying yeast
426	87	85½	Tallowy	Unclean, metallic	1,900,000	*Liquefying yeast

Samples 3 and 13 (duplicate). Raw cream (53°) churned at once, casein added

9	87	91	Old cream, moldy	Old cream	3,500,000	Lactic, *Oidium, non-liquefying yeast, *liquefying yeast
48	89	90½	Old cream	Old cream	4,000,000	Lactic, *Oidium, *liquefying yeast
164	88½	85	Old cream	Fishy, rancid, moldy, unclean	2,600,000	Non-liquefying yeast
275	86½	85	Old cream, tallowy, metallic	Stale, dishrag, flat, metallic	2,750,000	Lactic, *liquefying yeast, non-liquefying yeast
426	87	87	Tallowy	Fishy, tallowy	690,000	*Liquefying yeast

Samples 4 and 14 (duplicate). Raw cream (53°) churned at once, fishy butter added

9	93	91	Old cream	Old cream	2,050,000	Lactic, *Oidium, *Cladothrix, *liquefying yeast
48	88½	89½	Old cream	Old cream, abnormal acid	5,000,000	Lactic, *Oidium, *liquefying yeast
164	89	85½	Old cream, fishy	Old cream, fishy	3,300,000	*Liquefying yeast
275	88	86	Old cream, tallowy	Old cream, fishy, tallowy	1,100,000	*B. subtilis, M. lactic citreus B. (var. no pell.)
426	87½	87	Tallowy	Fishy, tallowy	540,000	Lactic

\* Organisms which liquefy either gelatine or milk or both.

TABLE 1—Continued

AGE	SCORE		CRITICISMS OF JUDGES		COUNT OF COLONIES PER GRAM OF SAMPLE	FLORA ISOLATED
	Sample	Duplicate	Sample	Duplicate sample		
Samples 5 and 15 (duplicate). Raw cream (53°) churned at once, boric acid added						
days 9	91	91	Old cream	Old cream, weak flavor	7,750,000	Lactic, *Oidium, *liquefying and non-liquefying yeasts
48	88	88½	Old cream	Old, flat, cheesy	5,000,000	Lactic, *Cladothrix, Streptococcus lactis aureus
164	89	87	Old cream	Old cream, sickish, feverish, light salt	2,800,000	Lactic, *B. subtilis, non-liquefying yeast
275	86½	86½	Old cream, fishy	Old cream, unclean	1,300,000	Lactic, *M. lactis albus, var. D.
426	87½	87	Fishy	Tallowy	1,150,000	
Samples 6 and 16 (duplicate). Pasteurized, not ripened, churned at once, [control]						
Fresh						
9	89	87	Metallic	Metallic, sickish	155,000	*Bact. lactis Marshalli, var. B.
48	88	88	Old cream, metallic	Old cream, metallic	77,000	*B. mycoides, Bact. lactis salmonis, *B. subtilis
164	87	88	Old cream, metallic	Old cream	32,000	*B. subtilis
275	84½	86½	Old cream, fishy, unclean, metallic	Strong, acrid, metallic	45,000	Non-liquefying yeast, *B. lactis Harrisoni (var.)
426	85	86	Metallic	Metallic	17,000	*Cladothrix, *Ps. lactis Eurotas

Samples 7 and 17 (duplicate). Pasteurized, not ripened, acidified H<sub>2</sub>O in second washing

	87	91	Metallic Old cream	Metallic Old cream, metallic	51,000 22,500	*B. lactis Harrisoni (var.) *M. lactis aureus, *Old- ium, non-liquefying yeast, Galactococcus versicolor (var.), *Bact. lactis fluorescens Galact. versicolor, lactie, *B. subtilis Lacties, Galact. versicolor
9 48	87½	88½				
164	88	86½	Old cream, unclean, me- talic	Old cream, acrid, gritty	47,000	
275	87	87	Old cream, metallic	Strong, fishy, acrid, me- talic	16,500	
426	86½	86	Tallowy, metallic	Metallic	10,000	

Samples 8 and 18 (duplicate). Pasteurized cream, not ripened, casein added

	87	89	Metallic Old cream, metallic	Old Old cream, dishrag, moldy, metallic Old cream, insipid, acrid, mineral flavor	85,000 57,000 38,000 15,500 27,000	*Bact. lactis Marshalli, var. B. Galact. versicolor, *Strep- tococcus lactis citreus I. Lacties (and others) Lacties, *Ps. lactis Euro- tas, Strep. lactis citreus I. var. A. B. lactis nebulus
9 48	87 88½	89 89½				
164	88	87	Old cream, insipid, mealy, metallic	Old cream, dishrag, moldy, metallic		
275	87½	86	Old cream, strong, acrid, granular, mealy, me- talic	Old cream, insipid, acrid, mineral flavor		
426	86	86	Metallic	Tallowy, unclean, me- talic		

\* Organisms which liquefy either gelatine or milk or both.



TABLE 1—Continued

AGE	SCORE		CRITICISMS OF JUDGES		COUNT OF COLONIES PER GRAM OF SAMPLE	FLORA ISOLATED
	Sample	Duplicate	Sample	Duplicate sample		
Samples 9 and 19 (duplicate). Pasteurized cream, not ripened, fishy butter added						
days						
9	88	89	Old cream, metallic	Metallic	80,000	*Oidium, Bact. lactis sal-
48	88	89½	Old cream, metallic	Old cream, metallic	39,000	monis, Galact. versi-
164	87	86½	Old cream, strong, me-	Pasty, unclean, metallic	82,000	color, *liquefying yeast,
275	87	87½	talic, abnormal acid		15,000	*Bact. lactis fluorescens
426	85½	85½	Old cream, strong, me-	Metallic, heavy salt	27,000	Lactic (?), non-liquefying
			talic, heavy salt			yeast
			Metallic			Lactics, Bact. lactis Mar-
						shalli, var. B.
						Non-liquefying yeast, *Ps.
						lactis Eurotas, *Ps.
						lactis Eurotas (var. no
						pellicle), *Ps. lactis Eu-
						rotas (rapid liquefying)
Samples 10 and 20 (duplicate). Pasteurized cream, not ripened, boric acid added						
9	88	89	Old cream, metallic	Metallic	60,000	Bact. lactis Marshalli,
48	88	88½	Old cream, metallic,	Old cream, rancid, me-	40,000	var. B., B. lactis Col-
164	88	86½	heavy salt	talic	32,000	chesterii
275	87	88	Old cream, metallic,	Old cream, moldy un-	20,500	Bact. lactis Marshalli,
426	86½	87	gritty	clean, metallic	29,000	var. B.
			Old cream, strong, me-	Old cream, strong, me-		Bact. lactis Marshalli,
			talic	talic		var. B.
			Metallic	Tallowy		Bact. lactis Marshalli,
						var. B., *M. lactis vari-
						ans
						*Ps. lactis Eurotas

Samples 21 and 31 (duplicate). Raw cream (53°) ripened to 60° acid [control]

Fresh	90	88	Old cream, acid	Old cream, unclean	25,500,000	Lactic, *liquefying yeast.
48	88	88½	Old, unclean, acid, metallic	Old cream, old, metallic	10,500,000	Lactic, *Oidium, *liquefying yeast
164	86½	85	Fishy, acid, metallic	Fishy, unclean	2,900,000	Lactic, non-liquefying yeast
275	86	85	Old cream, acid, metallic	Strong, fishy, metallic	830,000	Lactic, *liquefying yeast, B. lactis citreus, B. lactis varians (var.)
426	86½	85	Fishy	Fishy, unclean	850,000	Lactic (?), *liquefying yeast
					100,000	Lactic

Samples 22 and 32 (duplicate). Raw cream (53° acid), ripened to 60° acid, washed in acidified water

9	89	87	Cheesy, acid	Old cream, unclean	9,050,000	Lactic, *Oidium, *liquefying yeast, M. lactis rosaceus (var.)
48	86½	87½	Old, fishy	Foreign, abnormal acid, metallic, heavy salt	4,600,000	Lactic, *Oidium, *liquefying yeast, *B. subtilis
164	86½	86½	Acrid, gritty, metallic	Acrid, gritty	2,500,000	Lactic, *liquefying yeast
275	87	89	Old cream, tallowy, acrid, metallic	Old cream, good flavor	1,600,000	Lactic (?), *liquefying yeast, Galact. versicolor
426	86½	86		Acrid, metallic	1,000,000	Lactics

\* Organisms which liquefy either gelatine or milk or both.

TABLE 1—Continued

AGE	SCORE		CRITICISMS OF JUDGES		COUNT OF COLONIES PER GRAM OF SAMPLE	FLORA ISOLATED
	Sample	Duplicate	Sample	Duplicate sample		
Samples 23 and 33 (duplicate). Raw cream (53° acid), ripened to 60° acid, casein added						
<i>days</i>						
9	91	89	Old cream	Old cream, tallowy, unclean	8,800,000	Lactic, *Oidium, *liquefying yeast
48	89	88½	Old	Old cream, metallic	4,250,000	Lactic, *B. subtilis, Bact. lactis cretaceum
164	85	88½	Old cream, fishy, unclean, metallic	Old cream, fishy	2,500,000	Lactic, *liquefying yeast, non-liquefying yeast, Bact. lactis cretaceum
275	86	86½	Fishy, acrid, mealy, mineral flavor	Fishy, tallowy, metallic	2,550,000	Galact. versicolor
426	87	85½	Fishy	Fishy, metallic	1,827,000	*Liquefying yeast
Samples 24 and 34 (duplicate). Raw cream (53° acid), ripened to 60° acid, fishy butter added						
9	92	87	Old cream, tallowy	Old cream, old, stale	3,650,000	Lactic, *Oidium
48	89½	88½	Old cream, metallic	Old cream, old, unclean	3,250,000	Lactic, *Oidium, *Bact. lactis fluorescens, M. lactis citreus B (var. no pell.), S. lactis citreus I. (var. A.)
164	85	87	Old cream, fishy, unclean, metallic	Fishy	480,000	Lactic, *Oidium
275	85	89	Old cream, strong, fishy	Old cream, good flavor	170,000	Lactic
426	87	86½	Fishy	Fishy, metallic	1,047,000	Non-liquefying yeast

Samples 25 and 35 (duplicate). Raw cream (53°+), ripened to (60°+), boric acid added					
9	92	92	Old cream	Old cream	Non-liquefying <sup>*</sup> yeast, *Oidium
48	90½	88½	Old cream, good flavor, acrid	Weak flavor, unclean	Lactic, *Oidium, *liquefying yeast, *M. lacticis variants, M. lacticis variants A. (var.)
164	85	88	Old cream, fishy, moldy, metallic, unclean	Old cream, fishy	Lactic, *liquefying yeast, B. lacticis citreus
275	84	86	Old cream, strong, fishy	Strong, fishy, flat	Lactic
426	85½	86½	Fishy	Fishy, metallic, light salt	Lactic
Samples 26 and 36 (duplicate). Pasteurized cream (53° acid), ripened to 64°+ [control]					
Fresh	87	89	Metallic	Metallic	Lactics
48	87	87	Old, unclean, metallic	Dishrag, metallic	Small white lactic
164	86½	85½	Old cream, metallic	Old, woody, moldy	Lactic
275	86½	85½	Old cream, flat, metallic	Old cream strong, fishy, acrid, mealy, metallic	Lactic
426	85½	86	Metallic	Fishy, metallic	Lactic, Bact. lacticis Marshalli, var. B.
Samples 27 and 37 (duplicate). Pasteurized cream, ripened to 64°, washed in acidified H <sub>2</sub> O					
9	90	92	Old cream, acrid, metallic	Old cream, metallic	Lactics
48	86½	86½	Old, unclean	Abnormal acid, metallic	Lactics
164	87½	87½	Old cream, acrid, metallic	Old cream, metallic	Lactics, non-liquefying yeast
275	87½	86	Old cream, strong, acrid	Fishy, flat, acrid, mealy	Non-liquefying yeast
426	86	87	Acrid, metallic	Metallic	320,000

\* Organisms which liquefy either gelatine or milk or both.

TABLE 1—*Concluded*

AGE	SCORE		CRITICISMS OF JUDGES		COUNT OF COLONIES PER GRAM OF SAMPLE	FLORA ISOLATED
	Sample	Duplicate	Sample	Duplicate sample		
Samples 28 and 38 (duplicate). Pasteurized cream, ripened to 64°, casein added						
<i>days</i>						
9	87	89	Tallowy, metallic	Metallic	700,000	Lactic
48	88	88	Old cream, metallic	Old cream, metallic	270,000	Lactics
164	86½	87	Acrid, metallic	Strong, acrid	330,000	Lactics
275	86	86½	Strong, acrid, metallic	Strong, fishy, acrid, metallic	86,000	Lactics, <i>M. lactis citreus</i> (var., no pell.)
426	85	87	Metallic	Metallic	273,000	
Samples 29 and 39 (duplicate). Pasteurized cream, ripened to 64°+, fishy butter added						
9	88	89	Old cream, metallic	Old cream, metallic	700,000	Lactic
48	88½	89	Old, fishy, tallowy, unclean	Old cream, metallic	210,000	Lactic
164	86½	86½	Acrid, metallic	Unclean, acrid, metallic	31,000	Lactic
275	86½	86½	Old cream, flat, acrid, metallic	Strong, acrid	28,000	Bact. lactis Marshalli, var. B.
426	85½	86	Metallic	Metallic	167,000	* <i>Ps. lactis Eurotas</i>
Samples 30 and 40 (duplicate). Pasteurized cream, ripened to 64°+, boric acid added						
9	87	90	Tallowy, metallic	Metallic	540,000	Lactic
48	88½	88	Old cream, metallic	Old cream, abnormal acid, metallic	210,000	Lactic, * <i>Oidium</i>
164	85½	86½	Acrid, metallic	Unclean, acrid	200,000	Lactic
275	87	86½	Old, flat, tallowy, acrid	Metallic	280,000	Lactic * <i>M. lactis varians</i>
426	86½	87	Strong, metallic		112 000	

\* Organisms which liquefy either gelatine or milk or both.

Only two possible reasons have suggested themselves to the writers. One is that possibly the association with other bacteria may in some way prolong their activity. The other is that the fat may exert a protective influence by shielding certain favorably placed cells (those in the fat itself, not in the curd) from the acid produced by the cells. The low temperature and the salt too may cause such a slow rate of metabolism that relatively little acid is produced and so the life of the cell prolonged.

The most frequent species observed aside from the lactics were a liquefying and a non-liquefying yeast and a species of *Oidium*. Both the liquefying yeast and the *Oidium* occur less frequently (in fact, only rarely) in the pasteurized cream butters than in the raw cream butters. This suggests that these organisms entered the cream largely before pasteurization and were killed off during the process. The few that occurred in the pasteurized cream butters may have come from unsterile butter utensils or from the air.

Every one of the butters except samples 26, 27, and 28 contained one or more and in some cases as high as six different species of liquefying organisms. These are indicated in the table by an asterisk. The presence of these organisms helps to explain the production of the soluble nitrogen noted below.

The salt agar plates incubated at room temperature contained relatively few bacteria, the counts being 9500 per gram, 2800 per gram, 8100 per gram, and 3100 per gram for samples I, VI, XXI, and XXVI, the butters which were examined when freshly made. At nine days the highest count obtained was 6300 per gram for sample IX. The numbers usually were below 3000 per gram for the other samples, with only a few hundred remaining at the end of the period of storage.

The salt agar plates incubated at 6°C. made a much poorer showing than those incubated at room temperature, the counts ranging from 0° to 2200 per gram.

The flora isolated from the salt agar plates contained practically the same predominant forms as appeared on the litmus lactose agar plates except that the lactics were completely repressed by the salt. A number of species not appearing on the

litmus lactose agar plates appeared on the salt agar plates but only in small numbers so that they are of no particular significance.

## ANALYTICAL DATA

The analytical data were secured in the hope that by their aid some of the changes taking place in the butter might be explained. Previous papers from this station had suggested that

TABLE 2  
*Per cent of moisture and of salt in the experimental butters*

NUMBER OF SAMPLE	MOISTURE				SALT					SALT IN MOISTURE
	Age of butter (days)			Aver- age	Age of butter (days)				Aver- age	
	11	50	166		11	50	166	277		
										<i>per cent</i>
1	13 95	13.74	14.09	13 93	2 40	2 45	2 50	2.27	2.51	18.0
2	13 27	13.54	13.12	13 31	3.55	3 60	3 55	4 00	3 65	27.4
3	14.03	14 00	14 39	14 14	2 40	2.40	2.45	2 20	2 36	16.7
4	14 07	13 95	14 08	14.03	2 40	2 50	2 45	2 55	2.47	17.6
5	14 20	14 37	14.23	14.27	2 55	2 50	2 60	2 65	2.57	18 0
6	18 75	19.10	18 88	18 91	4 55	4 50	4 65	4 75	4.61	24 3
7	14 54	14 38	13.54	14 49	4 20	4.15	3 60	3 90	3.96	27.4
8	19 83	19 70	19.82	19 78	4 85	4.90	4 95	5 05	4 94	25 0
9	19.45	19 21	19.16	19 27	4 65	4 80	4 70	4 90	4 74	24.6
10	19 62	19.60	19.32	19.51	4 75	4 85	4 95	4.90	4 86	24 9
21	16 31	16 12	16 40	16 28	3 35	3 40	3 35	3 20	3 32	20 4
22	13.82	13 95	14 03	13.93	4 25	4 30	4 00	4 20	4 19	30 0
23	16 26	16.13	16.37	16.25	3 30	3.40	3 35	3.50	3.39	20 8
24	16.10	16.17	16.08	16.12	3 30	3 25	3 10	3.20	3.21	19.9
25	16 15	16 62	16 26	16 34	3 35	3 40	3 25	3 40	3 35	20.5
26	19 53	19.06	19 68	19.42	4.80	4.70	4.60	4.55	4.66	24.0
27	14 58	14 65	15.11	14.81	3.80	3 65	3.75	3.50	3.67	24.8
28	19 93	19.86	19 81	19 87	4.75	4 80	4.85	5.10	4 78	24.5
29	19.52	19.72	19.44	19.56	4.65	4.70	4 60	4.60	4.64	23.7
30	19 94	19 89	19.43	19.74	4 75	4 80	4 55	4 60	4.67	23.6

some of the off flavors were due to changes in the protein portion of the butter (5).

*Salt and moisture.* The salt and moisture determinations are recorded in table 2.

It will be seen that a number of the samples contained a high percentage of moisture this being especially true of the pasteurized samples. The same thing may be said of these samples in respect to the percentage of salt. In the last column are the calculated percentages of the strengths of brine in each butter.

TABLE 3  
*Lactose in the experimental butters*

NUMBER OF SAMPLE	PER CENT IN BUTTER					AS PER CENT OF MOISTURE IN BUTTER				
	Age of butter (days)					Age of butter (days)				
	At first	11	166	277	428	At first	11	166	277	428
1	0.30	0.29	0.28	0.27	0.27	2.16	2.08	2.02	1.94	1.94
2		0.28	0.29	0.28	0.28		2.14	2.21	2.12	2.12
3		0.31	0.29	0.29	0.29		2.18	2.05	2.05	2.05
4		0.30	0.28	0.28	0.28		2.14	2.00	2.00	2.00
5		0.30	0.29	0.29	0.28		2.10	2.02	2.02	1.96
6	0.32	0.29	0.27	0.26	0.26	1.70	1.54	1.43	1.38	1.38
7		0.33	0.32	0.28	0.28		2.32	2.25	1.97	1.97
8		0.28	0.26	0.27	0.26		1.41	1.31	1.36	1.31
9		lost	0.33	0.32	0.30		lost	1.71	1.65	1.56
10		0.31	0.27	0.30	0.29		1.59	1.39	1.54	1.49
21	0.33	0.29	0.28	0.29	0.29	2.02	1.78	1.72	1.78	1.78
22		0.30	0.29	0.30	0.29		2.16	2.08	2.16	2.18
23		0.33	0.32	0.31	0.31		2.02	1.91	1.91	1.91
24		0.30	0.29	0.30	0.30		1.87	1.81	1.87	1.87
25		0.33	0.31	0.31	0.31		2.02	1.90	1.90	1.90
26	0.31	0.30	0.28	0.28	0.28	1.60	1.54	1.45	1.45	1.45
27		0.29	0.29	0.27	0.28		1.96	1.96	1.83	1.89
28		0.30	0.29	0.29	0.29		1.51	1.46	1.46	1.46
29		0.29	lost	0.29	0.29		1.48	lost	1.48	1.48
30		0.29	0.28	0.29	0.28		1.47	1.42	1.47	1.42
Average	0.315				0.2855	1.87				1.756

This makes the high bacteriological counts already discussed all the more striking since most of these results show very strong brines, some of them being about two-thirds saturated solutions.

*Lactose.* In table 3 are shown the percentages of lactose in the butters and a recalculation of the results in terms of percentage of lactose in the moisture of the butter.



If the average of the amount of lactose in all of the butters when fresh is compared with the average at the age of 426 days it will be seen that there is a slight but unmistakable decline in the amount of lactose. The average when fresh is 0.315 per cent and when old is 0.2855 per cent, or in terms of the moisture

TABLE 4

*Acidity determinations in experimental butters (cubic centimeters of N/10 NaOH per 100 grams butter)*

NUMBER OF SAMPLE	ACIDITY OF BUTTER						ACIDITY WASHED FROM BUTTER					
	Age of butter (days)						Age of butter (days)					
	At first	11	50	166	277	428	At first	11	50	166	277	428
1	25	27	28	31	30	34	3 0	4.0	3.5	3.4	3.3	3.3
2		32	30	34	30	32		5.3	3.2	4.3	3.8	4.0
3		29	29	34	32	36		4.8	3.6	4.0	3.9	3.5
4		28	29	32	30	33		4.9	3.6	3.8	3.7	3.7
5		30*	24*	25*	28*	23*		7.0*	6.8*	4.4*	4.5*	3.8*
		(50)	(44)	(45)	(48)	(43)		(9.0)	(8.8)	(6.4)	(6.5)	(5.8)
6	18	20	17	20	21	20	2 1	2.7	2.4	2.1	1.8	2.0
7		22	21	22	24	22		3.0	2.6	2.9	3.1	3.1
8		20	19	19	20	20		2.7	2.5	2.5	2.6	2.6
9		18	18	19	20	18		2.6	2.5	2.7	2.1	2.1
10		20*	17*	23*	25*	27*		6.7*	5.7*	3.9*	4.0*	3.5*
		(35)	(32)	(38)	(40)	(42)		(8.2)	(7.2)	(5.4)	(5.5)	(5.0)
21	26	32	30	33	31	30	4 0	4.3	4.3	4.9	4.1	3.7
22		32	31	36	32	38		4.6	4.3	4.3	3.2	3.2
23		32	28	34	32	30		5.0	4.8	4.5	4.5	4.1
24		32	32	38	37	38		5.7	6.1	5.5	4.5	5.7
25		32*	30*	33*	32*	31*		6.4*	6.2*	5.6*	6.4*	7.2*
		(45)	(43)	(46)	(45)	(44)		(7.7)	(7.5)	(6.9)	(7.7)	(8.5)
26	20	20	20	21	20	20	2.4	3.1	2.4	3.0	2.7	3.2
27		21	23	24	24	22		3.7	2.3	3.5	3.1	2.4
28		20	20	22	25	24		3.8	2.2	3.2	3.0	2.2
29		21	22	23	24	22		4.1	3.6	3.0	2.9	2.5
30		31*	24*	26*	22*	24*		7.4*	5.6*	3.7*	3.4*	3.2*
		(49)	(42)	(44)	(40)	(42)		(9.2)	(7.4)	(5.5)	(5.2)	(5.0)
Average	322.5	27.2					32.875	3.95				

\* Acidity due to boric acid added is not included.

in the butter, the figures become 1.87 and 1.756 per cent, respectively. This, no doubt, was used up in producing acid. The smallness of the amount used (viz., 0.095 per cent) for the production of acid supports the theory expressed on a previous page that the metabolism of the acid forming bacteria was retarded by the presence of the salt and the low temperature of storage.

*Acidity.* The determinations of the acidity of the butters are presented in table 4. As is to be expected, the acidity increased during the period of storage. The average number of cc. of N/10 soda per 100 grams of butters when fresh was 22.5, and after 426 days of storage was 27.2, a difference of 4.7 cc. Expressed in terms of acid washed from butter, representing soluble acid, the figures become 2.875 cc. for the average of the fresh butters, and 3.95 cc. for the average of the old butters, a difference of 1.075 cc. This is not very much but it is many times the amount that could have come from the average amount of lactose used up. This suggests that there was some hydrolysis of the fat or protein resulting in compounds capable of using up alkali. No other explanation except the unwelcome one of errors in technique suggests itself.

#### DEGRADATION OF THE NITROGEN

The total nitrogen for the various butters at the age of 11 and 166 days are given in table 5. The first three columns give the results calculated as per cent of the butter and the last two columns, as per cent of the moisture in the butter.

The most striking thing about these results is the fact that the pasteurized samples contained about one half of the nitrogen that was contained in the raw butters. These are similar to the results obtained by Mortensen, Gaessler, and Cooper (6), who found that when the cream was pasteurized in the sour condition, the per cent protein of the resulting butter was decreased over raw cream butter but that when sweet cream was pasteurized the protein content of the butter was not influenced. They offer the explanation that in sour cream "the casein in the presence

of acid is hardened and thrown into clumps known as curd particles, that are quite readily removed in the cleaning and washing of the butter." Hunziker, Spitzer, Mills and Switzer (7) also note a tendency of pasteurization to lower the curd in butter,

TABLE 5  
*Total nitrogen in experimental butters*

NUMBER OF SAMPLE	AS PER CENT IN BUTTER		AS PER CENT OF MOISTURE IN BUTTER	
	Age of butter (days)		Age of butter (days)	
	11	166	11	166
1	0.0756	0.0756	0.582	0.582
2	0.0812	0.0770	0.614	0.588
3	{ 0.0850* (0.1000)	0.0740* (0.0980)	{ 0.600* (0.769)	0.522* (0.683)
4	0.0812	0.0792	0.580	0.565
5	0.0798	0.0826	0.556	0.578
6	0.0518	0.0490	0.274	0.259
7	0.0504	0.0490	0.355	0.346
8	{ 0.0478* (0.0756)	0.0492* (0.0770)	{ 0.242* (0.381)	0.248* (0.389)
9	0.0476	0.0462	0.247	0.239
10	0.0476	0.0462	0.245	0.237
21	0.0868	0.0854	0.531	0.524
22	0.0840	0.0770	0.605	0.555
23	{ 0.0842* (0.1120)	0.0830* (0.1108)	{ 0.518* (0.699)	0.509* (0.685)
24	0.0854	0.0840	0.530	0.522
25	0.0868	0.0854	0.532	0.524
26	0.0560	0.0546	0.288	0.282
27	0.0588	0.0546	0.396	0.370
28	{ 0.0537* (0.0924)	0.0523* (0.0910)	{ 0.270* (0.465)	0.264* (0.457)
29	0.0532	0.0490	0.272	0.250
	0.0504	0.0476	0.256	0.242

but offer no explanation for the fact, though their explanation for loss of fat in the buttermilk would apply to the loss of curd as well; since they also postulate a shrinking of the curd in the presence of high acid and high heat.

Another explanation that may account for the lesser amounts of protein in the pasteurized cream butters is that the presence

of acid and heat may have resulted in a hydrolysis of a portion of the casein which would then be lost during the subsequent washing of the butters since it would then be soluble.

The nitrogen not precipitated by copper sulphate appears in table 6 where it is recorded both as percentage of the butter

TABLE 6  
*Nitrogen not precipitated by copper sulfate*

NUMBER OF SAMPLE	AS PER CENT OF BUTTER						AS PER CENT OF MOISTURE IN BUTTER					
	Age of butter (days)						Age of butter (days)					
	At first	11	50	166	277	428	At first	11	50	166	277	428
1	0.0060	0.0064	0.0064	0.0068	0.0071	0.0064	0.0432	0.0461	0.0461	0.0488	0.0510	0.0461
2		0.0063	0.0063	0.0059	0.0067	0.0067		0.0481	0.0481	0.0454	0.0511	0.0511
3		0.0064	0.0064	0.0064	0.0071	0.0064		0.0450	0.0450	0.0450	0.0499	0.0486
4		0.0060	0.0060	0.0064	0.0071	0.0064		0.0429	0.0429	0.0457	0.0502	0.0457
5		0.0060	0.0060	0.0060	0.0071	0.0064		0.0420	0.0420	0.0420	0.0492	0.0447
6	0.0031	0.0031	0.0031	0.0027	0.0035	0.0038	0.0164	0.0164	0.0164	0.0144	0.0185	0.0202
7		0.0030	0.0030	0.0026	0.0030	0.0030		0.0211	0.0211	0.0183	0.0211	0.0211
8		0.0031	0.0035	0.0031	0.0035	0.0035		0.0157	0.0177	0.0157	0.0181	0.0159
9		0.0027	0.0031	0.0027	0.0035	0.0035		0.0140	0.0161	0.0140	0.0181	0.0181
10		0.0027	0.0031	0.0027	0.0031	0.0031		0.0139	0.0159	0.0139	0.0159	0.0159
21	0.0064	0.0068	0.0064	0.0064	0.0076	0.0076	0.0392	0.0392	0.0417	0.0392	0.0465	0.0465
22		0.0056	0.0060	0.0056	0.0064	0.0060		0.0403	0.0432	0.0403	0.0460	0.0432
23		0.0064	0.0068	0.0064	0.0076	0.0072		0.0392	0.0416	0.0392	0.0465	0.0441
24		0.0064	0.0057	0.0061	0.0072	0.0072		0.0398	0.0355	0.0377	0.0448	0.0448
25		0.0061	0.0061	0.0057	0.0076	0.0068		0.0374	0.0374	0.0350	0.0464	0.0418
26	0.0035	0.0038	0.0035	0.0035	0.0038	0.0035	0.0180	0.0196	0.0180	0.0180	0.0196	0.0180
27		0.0034	0.0038	0.0034	0.0030	0.0030		0.0123	0.0157	0.0123	0.0206	0.0206
28		0.0039	0.0039	0.0039	0.0039	0.0039		0.0196	0.0196	0.0196	0.0196	0.0196
29		0.0039	0.0039	0.0035	0.0039	0.0039		0.0199	0.0199	0.0179	0.0199	0.0199
30		0.0031	0.0035	0.0031	0.0031	0.0035		0.0158	0.0177	0.0158	0.0158	0.0177

and as percentage of the moisture in the butter. This data has also been recalculated and recorded in table 7 as percentage of the total nitrogen.

As shown by table 7 there are only four exceptions (samples 5, 7, 26, and 27) to the rule that there was a higher percentage of soluble nitrogen at the end of the period of storage than when

the butters were fresh. In a general way this decomposition was progressive though there were many exceptions to the rule. There are also some cases where there was an apparent decrease in the percentage of soluble nitrogen. This, no doubt, was due to errors in technique or variation in the distribution of the

TABLE 7

*Nitrogen not precipitated by copper sulfate (recorded in per cent of total nitrogen)*

NUMBER OF SAMPLE	AT FIRST	11 DAYS	50 DAYS	166 DAYS	277 DAYS	428 DAYS
1	7.95	8.47	8.47	8.99	9.39	8.47
2		7.96	7.96	7.45	8.46	8.46
3		{ 8.05*	8.05*	8.05*	8.91*	8.55*
		(6.19)	(6.19)	(6.19)	(6.88)	(6.58)
4		7.48	7.48	7.98	8.85	7.98
5		7.39	7.39	7.39	8.74	7.88
6	6.15	6.15	6.15	5.36	6.94	7.54
7		6.04	6.04	5.23	6.04	6.04
8		{ 6.40*	7.20*	6.40*	7.21*	7.21*
		(4.07)	(4.58)	(4.07)	(4.58)	(4.58)
9		5.75	6.61	5.75	7.46	7.46
10		5.75	6.61	5.75	6.61	6.61
21	7.43	7.43	7.89	7.43	8.81	8.81
22		6.95	7.46	6.95	7.95	7.46
23		{ 7.58*	8.05*	7.58*	9.00*	8.51*
		(5.69)	(6.03)	(5.69)	(6.75)	(6.40)
24		7.55	6.73	7.20	8.50	8.50
25		7.00	7.00	6.55	8.27	7.82
26	6.34	6.87	6.34	6.34	6.87	6.34
27		6.00	6.71	6.00	5.29	5.29
28		{ 7.36*	7.36*	7.36*	7.36*	7.36*
		(4.26)	(4.26)	(4.26)	(4.26)	(4.26)
29		7.64	7.64	6.85	7.64	7.64
30		6.33	7.14	6.33	6.33	7.14

\* Nitrogen added to butter in form of casein is not included.

nitrogen compounds in the butters. The increase in soluble nitrogen as recorded in table 7 follows in a general way the decrease in total nitrogen as shown by table 5.

A curious fact may be noted in connection with these analyses. While the pasteurized cream butters contained only about one-

half of the amount of nitrogen as was contained in the raw cream butters, and the percentage of soluble nitrogen was also lower in the pasteurized butters than in the raw butters, the percentage of the total nitrogen which was rendered soluble was about the same in both types of butters. The explanation for this fact is not clear (though it probably could be explained by the law of mass action) since "it is known that protein may hydrolize on long standing, in the presence of water alone without the assistance of any intermediary agents" (8).

#### SUPPLEMENTARY WORK ON THE ACTION OF THE BUTTER FLORA ON MILK

Supplementary to the work outlined above some additional work was done with some of the typical butter micro-organisms to study their individual action upon milk. It is known that the micro-organisms most commonly found in butter are to a great extent those bacteria that were present in the milk and cream from which the butter was made; therefore, the initial butter flora is milk flora and vice versa. The changes taking place in milk can be more easily studied since they proceed with greater rapidity than the changes taking place in butter. Only the possibility of growth in milk and the action upon the nitrogenous constituents will be considered here.

The casein in butter during storage is slowly broken down into amino-acids and ammonia. Nitrogen, as amino-acids and ammonia, in percentage of the total nitrogen in unsalted butter (average of tubs from the creameries) was found in a previous study (9) to be 5.71 per cent at first and 7.59 per cent after 240 days in storage at 21°F.; in salted butter from the same three churnings and held in the same storage, it was found to be 5.71 per cent at first and 8.19 per cent after 240 days. And again as shown in the present study (see table 7) in salted butter made from pasteurized and from unpasteurized cream, the percentage (average of 20 tubs) increased from 6.24 per cent to 6.86 per cent for the pasteurized and from 7.68 per cent to 8.25 per cent for the unpasteurized butter during storage at 0°F. for 428 days.

Milk was found by M. E. Pennington (10) to contain certain species of bacteria especially resistant to cold; these became numerous and frequently occurred in almost pure culture after five to six weeks at 0.55°C. As a rule the liquefying group increased in proportion to the acid forming group. The casein of the milk in cold storage was rapidly digested until more than 50 per cent was changed into soluble compounds. Working with milk at low temperature Ravenel, Hastings and Hammer (11) give data showing that milk held at 0°C. for 203 days increased markedly in bacterial content which resulted in a rise in acidity to 0.70 per cent and a change in the casein rendering 70 per cent of the total nitrogen soluble. In milk held at 0°C., there was no increase in bacterial count, a slight decrease in acidity and 20 per cent of the total nitrogen was soluble after 203 days.

When the growth in plain milk of twelve different bacteria isolated from storage butter was compared with their growth in milk to which had been added 5 per cent sterile salt, it was observed (table 8) that while all of these bacteria were capable of making a good growth on agar containing 12 per cent salt—seven grew well on 16 per cent—the addition of 5 per cent salt to milk had a slight retarding effect. One of the organisms, however, (*M. l. citreus*) grew as well or even made a better growth in salted milk. The number added per cubic centimeter of milk taking the average for the twelve different organisms was 2,322 which increased in plain milk during one, three, and seven days at 20°C. to 140,000, 12,860,000 and 80,650,000, respectively, and in the milk plus 5 per cent salt to 113,800, 5,743,000 and 14,924,000, respectively. As none of these organisms form more than a trace of acid the acidity of the milk showed only a slight increase, the average acidity after one, three and seven days being 0.18 per cent, 0.20 per cent and 0.20 per cent for plain milk and 0.19 per cent, 0.21 per cent and 0.20 per cent for the milk plus 5 per cent salt.

The action exerted by these bacteria upon the casein was influenced by the presence of salt very much in the same way as the rate of growth was influenced, that is, where the rate of

TABLE 8  
*Bacterial count and acidity*

ORGANISM	AT FIRST		1 DAY		3 DAYS		7 DAYS	
	Acid	Count	Acid	Count	Acid	Count	Acid	Count
Milk without salt at 20°C.								
B. lactis Harrisoni..	0.10	10	0.15	10,000	0.22	3,000,000	0.32	1,160,000
Bact. lactis creta- ceum .....	0.16	3,000	0.15	100,000	0.17	75,000,000	0.07	120,000,000
B. subtilis .....	0.16	1,185	0.17	10,000	0.17	2,250,000	0.17	17,000,000
B. mycoides .....	0.16	49	0.19		0.21	3,000,000	0.26	1,000,000
S. lactis citreus 1...	0.16	17,500	0.18	833,000	0.18	14,450,000	0.15	162,000,000
M. lactis varians A.	0.16	26	0.18	50,000	0.18	1,000,000	0.20	
(?) .....	0.16	1	0.17	5,000	0.19		0.19	
M. lactis albus var. D .....	0.18	410	0.20	100,000	0.19		0.29	414,000,000
Ps. lactis Eurotas ..	0.18	685	0.20	250,000	0.21	950,000	0.22	7,250,000
M. lactis rubidus...	0.18	30	0.20		0.20			
M. lactis citronus...	0.18	4,310	0.20	41,500	0.20	4,260,000	0.22	3,350,000
B. (?) .....	0.18	675	0.21	1,000	0.22	40,000		120,000
Average .....	0.17	2,322	0.18	140,000	0.20	12,860,000	0.20	80,650,000

Milk with 5 per cent salt at 20°C.

B. lactis Harrisoni..	0.16	10	0.14	1,000	0.20	100,000	0.17	580,000
Bact. lactis creta- ceum .....	0.16	3,000	0.14	100,000	0.19	30,000,000	0.16	26,000,000
B. subtilis .....	0.16	1,185	0.18	10,000	0.19	1,400,000	0.21	23,000,000
B. mycoides .....	0.16	49	0.19	1,000	0.23	550,000	0.25	2,000,000
S. lactis citreus 1...	0.16	17,500	0.19	735,000	0.20	9,400,000	0.17	41,000,000
M. lactis varians A.	0.16	26	0.20		0.20		0.20	
(?) .....	0.16	1	0.18	5,000	0.20		0.19	
M. lactis albus var. D .....	0.18	410	0.20	30,000	0.19	130,000	0.21	320,000
Ps. lactis Eurotas ..	0.18	685	0.20	150,000	0.22	685,000	0.22	2,050,000
M. lactis citronus...	0.18	30	0.21	105,000	0.24	9,500,000	0.21	227,540,000
M. lactis rubidus...	0.18	4,310	0.20		0.22		0.20	
B. (?) .....	0.18	675	0.22	1,000	0.24	20,000	0.24	
Average .....	0.17	2,322	0.19	113,800	0.21	5,743,000	0.20	14,924,000



growth was greatest the action was most marked (see tables 9 and 10). This group of twelve bacteria is capable of transforming casein into caseoses and caseones and then into amino-acids and ammonia, although with some of the organisms it appears from

TABLE 9  
*Nitrogen as caseoses and caseones (per cent of total milk)*

ORGANISM (AT 20°C.)	MILK WITHOUT SALT			MILK WITH 5 PER CENT SALT		
	1 day	3 days	7 days	1 day	3 days	7 days
<i>B. lactis Harrisoni</i> . . . . .	0 023	0 072	0 087	0 028	0 029	0.069
<i>Bact. lactis cretaceum</i> . . . .	0 023	0 028	0.096	0 024	0 029	0.046
<i>B. subtilis</i> . . . . .	0 027	0 028	0 040	0 026	0.028	0 029
<i>B. mycoides</i> . . . . .	0 032	0.046	0 048	0 031	0 033	0 050
<i>S. lactis citreus</i> 1 . . . . .	0 029	0 031	0 042	0 028	0 029	0.036
<i>M. lactis varians</i> A . . . . .	0 026	0 032	0 036	0 028	0 034	0.034
(?) . . . . .	0 029	0 029	0 069	0 034	0 034	0.034
<i>M. lactis albus</i> var. D . . . .		0.030	0 037		0 035	0.034
<i>Ps. lactis Eurotas</i> . . . . .	0 035	0 035	0 036	0.035	0 038	0.038
<i>M. lactis rubidus</i> . . . . .	0 036	0 037	0 042	0 038	0 039	0.046
<i>M. lactis citronus</i> . . . . .	0 035	0.035	0 074	0 038	0.039	0.044
<i>B. (?)</i> . . . . .	0 043	0.042	0.046	0.042	0.042	0.046
Average . . . . .	0.031	0.037	0.054	0.030	0.034	0.042

TABLE 10  
*Nitrogen as amino-acids and ammonia (per cent of total milk)*

ORGANISM (AT 20°C.)	MILK WITHOUT SALT			MILK WITH 5 PER CENT SALT		
	1 day	3 days	7 days	1 day	3 days	7 days
<i>B. lactis Harrisoni</i> . . . . .	0.022	0 072	0 200	0.018	0.024	0.024
<i>Bact. lactis cretaceum</i> . . . .	0 023	0 026	0.107	0.019	0.023	0 025
<i>B. subtilis</i> . . . . .	0.028		0.041	0.022	0.026	0.023
<i>B. mycoides</i> . . . . .	0.031	0.072	0.130	0.029	0.043	0.082
<i>S. lactis citreus</i> 1 . . . . .	0.029	0 031	0.054	0.025	0 028	0.042
<i>M. lactis varians</i> A . . . . .	0.027	0.034	0.034	0.030	0.035	0.035
(?) . . . . .	0.026	0.034	0.106	0.027	0 031	0.033
<i>M. lactis albus</i> var. D . . . .	0.037	0.034	0.038	0.029	0.034	0.036
<i>Ps. lactis Eurotas</i> . . . . .	0.037		0.038	0.035	0.033	0.041
<i>M. lactis rubidus</i> . . . . .	0.037	0.038	0.039		0.038	0.046
<i>M. lactis citronus</i> . . . . .	0.037		0.079	0.037	0.036	0.043
<i>B. (?)</i> . . . . .	0.042	0.042	0.050	0.040	0.042	0.045
Average . . . . .	0.031	0.042	0.076	0.028	0.035	0.041

our few analyses that amino-acids and ammonia increase as soon as the action upon the casein begins. Products of the first stages of casein decomposition were found after one, three, and seven days in the following amounts (average for the twelve bacteria) 0.031 per cent, 0.037 per cent and 0.054 per cent for plain milk and 0.030 per cent, 0.034 per cent and 0.042 per cent for the milk with 5 per cent salt. Amino acids and ammonia appeared as follows 0.031 per cent, 0.042 per cent, and 0.076 per cent for plain milk and 0.028 per cent, 0.035 per cent and 0.041 per cent salted milk. If plotted the curves for the changes in salted milk would be found to be the same in nature as those for the plain milk but of a lower magnitude.

#### CONCLUSIONS

While the results of this study failed to reveal any striking proofs of the relationship between methods of manufacture and either the score or the development of definite off-flavors, yet certain facts stand out sufficiently well to warrant mention. They are as follows:

The raw cream butters quickly developed the old cream flavor which was later followed by fishy flavor, while the pasteurized (sour) cream butters early developed a metallic flavor. Tallowy flavor developed more frequently in the raw cream butters than in the pasteurized cream butters. Acid flavor developed much more frequently in well ripened pasteurized butters than in raw butters. From this study there is no evidence that either pasteurization or ripening improves the keeping quality of butters made from cream which has already soured.

Relatively higher bacteriological counts were obtained on butters over a year old than are usually obtained in studies of this kind, though there was a fairly rapid dying off in members at first.

The lactic acid bacteria appeared on the plates for a longer period than is usually thought possible, though there was a gradual displacement of the lactic acid flora by a more miscellaneous flora, among which the predominant types were a liquefying and a non-liquefying yeast and an oidium species.

Taking the butters as a whole, there was a slow gradual decrease in the amount of lactose present and a gradual increase in acidity, though the decrease of the one was not proportional to the increase in the other.

The amount of nitrogen in the pasteurized cream butters was about half that of the raw cream butters, but the percentage of nitrogen in soluble form was about the same in each class of butters, though it was very small in either case.

When 12 of the typical butter organisms were inoculated into milk alone and milk containing 5 per cent of salt, it was found that the number of bacteria increased rapidly in both cases but more rapidly when salt was not present. The increase in acidity was very slight in either case and of about the same amount. The action of these organisms upon the nitrogenous compounds of milk without and with the addition of 5 per cent salt was to increase the amount of soluble nitrogen in both cases but more so without than with salt. This amount of salt, however, does not retard the growth and action of these butter organisms as much as might seem possible.

#### REFERENCES

- (1) BENEDICT, STANLEY R.: Detection and estimation of sugars. *Journal of Biological Chemistry*, iii, 101-117, 1907.
- (2) SUPPLEE, GEORGE CORNELL: The lecithin content of butter and its possible relationship to the fishy flavor. Cornell University Agricultural Experiment Station, Memoir No. 29, 97-151, 1919.
- (3) HUNZIKER, O. F., AND HOSMAN, D. FAY: Tallowy butter—its causes and prevention. *Journal of Dairy Science*, i, 320-346. 1917.
- (4) PALMER, L. S., AND COMBS, W. B.: Relation of the enzymes of butter to the production of tallowiness by copper salts and over neutralization. *Jour. of Dairy Science*, ii, 444-452, 1919.
- (5) SAYER, WM. S., RAHN, OTTO, AND FARRAND, BELL: Keeping qualities of butter. I. General Studies. Mich. Agricultural College Experiment Station Tech. Bul. No. 1, 3-61, 1908.  
RAHN, OTTO, BROWN, C. W., AND SMITH, L. M.: Keeping qualities of butter. II. The influence of salt. III. The decomposition of proteins. Mich. Agricultural College Experiment Station, Tech. Bul. No. 2, 3-44, 1909.
- (6) MORTENSEN, M., GAESSLER, W. G., AND COOPER, W. H.: The pasteurization of cream for butter making. Part I. Effect on quality and chemical composition. Iowa Agricultural Experiment Station, Bul. No. 156, 3-26, 1914.

- (7) HUNZIKER, O. F., SPITZER, GEORGE, MILLS, H. C., AND SWITZER, H. B.: Pasteurization of sour farm-skimmed cream for butter making. Purdue University Agricultural Experiment Station, Bul. 208, 3-76, 1912. See also Hunziker, O. F., Mills, H. C., and Spitzer, George: Moisture control of butter, II. Purdue University Agricultural Experiment Station, Bul. No. 160, 365-419. 1912. Last two paragraphs on p. 376 discuss loss of fat due to pasteurization.
- (8) See p. 38 of the publication by Hunziker et al. noted in first reference of No. 7.
- (9) See second reference under 5.
- (10) PENNINGTON, M. E., HEPBURN, J. S., ST. JOHN, E. Q., WITMER, E., STAFFORD, M. O., AND BURRELL, J. I.: Bacterial and enzymic changes in milk and cream at 0°C. Jour. of Biological Chemistry, xvi, 331-368, 1913.
- (11) RAVENEL, M. P., HASTINGS, E. G., AND HAMMER, B. W.: The bacterial flora of milk held at low temperatures. Jour. Infect. Dis., iii, 38-46. 1910.

# AN ASSOCIATIVE STUDY OF STREPT. LACTICUS AND B. SUBTILIS IN MILK

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There is well-known evidence in the literature (1) of bacteriology that concerns the growth of several microorganisms together in the same medium. In such associative growth the development of each organism is likely to be influenced by the development of the others; the life curve of each will probably vary from the natural life curve of the organisms in pure culture. Since milk ordinarily contains a comparatively large number of microorganisms of various species, and is both an excellent food and a liquid, thereby offering a maximum chance for diffusion, it would seem that the natural interrelations of associative development would be as evident in milk as anywhere. Thus, with the purpose of studying the possibilities of associative growth in the lactic acid fermentation in milk, a typical strain of *Strept. lacticus*, isolated from the local dairy starter, and *B. subtilis*, obtained from the American Museum of Natural History, were grown together.

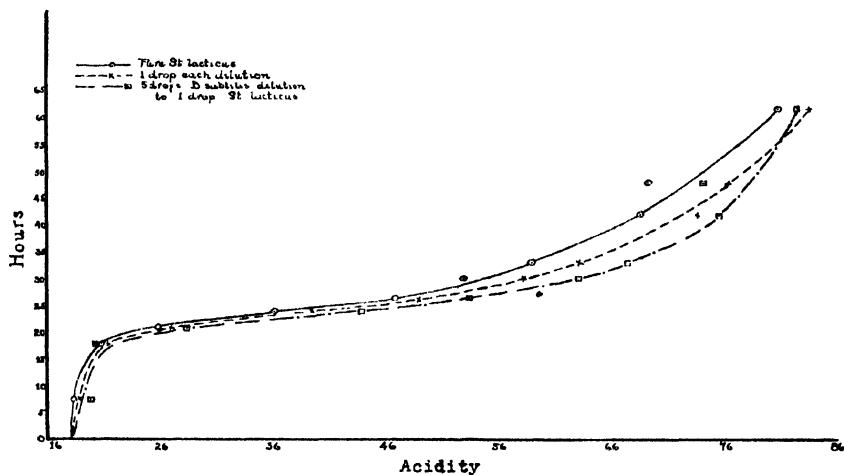
The general procedure apart from variations made for especial details was simple. Milk cultures of the two organisms from twenty-four to forty-eight hours old were used. Dilutions were made from these from which 100 cc. lots of sterile litmus milk were inoculated with varying quantities of the two organisms as desired, using as a unit of the dilution a drop from a Roux pipette. The flasks were kept at room temperature and the developments watched by means of the reduction and oxidation of the litmus and by means of titrations with phenolphthalein. The interest was, of course, focused on the variation in lactic fermentation in associative cultures as contrasted with controls of the pure lactic streptococci.

It was first evident that the presence of *B. subtilis* in milk did stimulate the lactic fermentation (graph 1). It will be seen from the graph that at any time during the most rapid lactic fermentation that associative cultures give a higher acidity than the control, and furthermore the variance from the control is proportional to a function of the original concentration of *B. subtilis*. It may be said that the associative influence can scarcely be looked for as increasing beyond 54°, Fuller's scale, at the latest, since the *B. subtilis* can not develop beyond this acidity. The relationships represented in these curves have been brought out repeatedly, always with the same results. Hence the presence of *B. subtilis* in milk is likely to stimulate the lactic fermentation.

Knowing the relative courses of these cultures in fermentation, it was desired to look into the numerical changes which were taking place during the fermentation. The results (graph 2) were the best that could be obtained by the plating method.

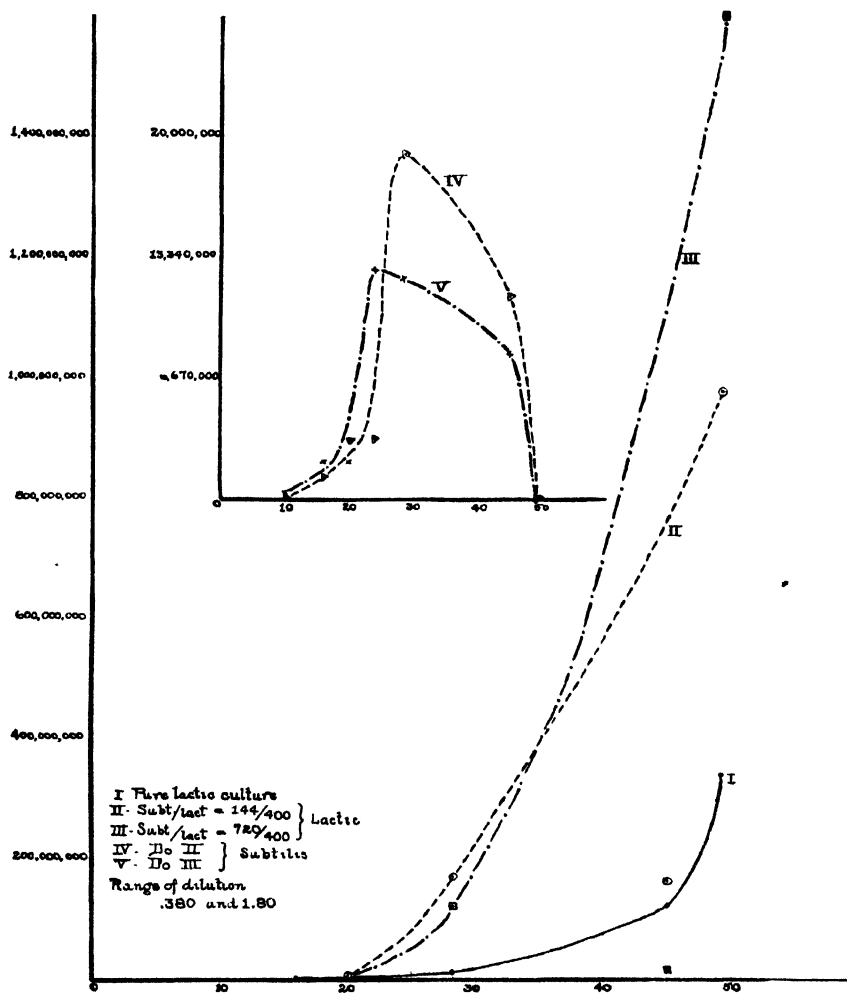
TIME	LACTIC	1 : 1 (DROPS)		5 : 1 (DROPS)	
		Subtilis	Lactic	Subtilis	Lactic
<i>hrs.</i>					
10.0		82,500		319,000	
16.0	65,500	1,230,000		2,050,000	
20.0	406,000	3,270,000	290,000	2,030,000	380,000
24.0		3,180,000		12,720,000	
28.2	11,000,000	19,000,000	170,000,000	12,000,000	122,000,000
45.0	126,000,000	11,000,000	161,000,000	8,270,000	19,080,000
49.2	343,000,000	None	980,000,000	None	1,601,000,000

Counting these became a difficult process since both organisms were present of necessity in the same plate. It will be observed that at fifty hours, however, there were no more organisms of *B. subtilis* so that the last points on the streptococcus curves should be fairly accurate. There is a numerical rise in the *Strept. lacticus* coinciding with the fermentation, as could only be expected. The *B. subtilis* shows a rapidly increasing rise up to a certain point when the lactic fermentation gains the ascendancy. It is important to note that the culture receiving originally the



Relative Rate of Rise in Acidity  
of *Strept. lacticus* as associated with *B. subtilis*.

Hours	Acidity		
	Lactic	1:1	5:1
0	18.0	18.0	18.0
7.5	18.3	18.6	19.6
18.0	20.7	21.3	20.3
21.0	25.7	26.7	26.3
24.0	36.0	39.3	43.7
26.2	46.7	48.7	53.3
30.0	52.7	58.3	63.0
33.0	58.7	63.0	67.3
42.0	68.3	73.3	75.3
48.0	69.0	76.0	73.7
62.0	80.2	83.0	82.0



Numerical Rise of Normal and Associative Cultures

Graph No. 2

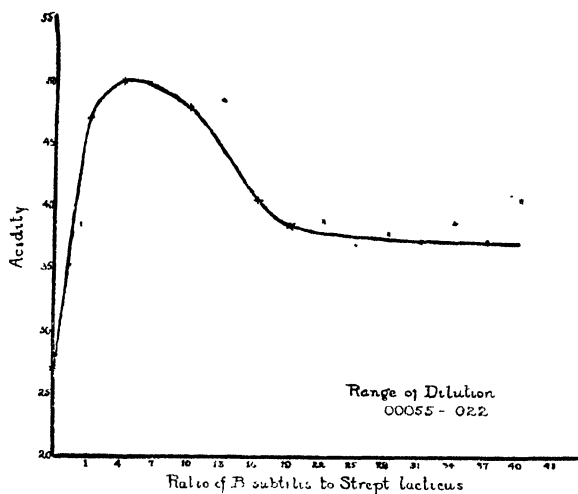


fewer organisms of *B. subtilis* of the two associative cultures (line IV) reaches a higher concentration than the culture receiving originally more organisms (line V).

There is theoretical evidence, therefore, of an "optimum" initial ratio of *B. subtilis* to *Strept. lacticus*, and this has actually been demonstrated (graph 3). Unfortunately it can not be repeated at will, for only by chance can the proper ratio be obtained. In this graph are considered at a certain time the various stages of fermentation in cultures inoculated with different ratios of the two organisms, using the acidity as an indicator to the differences. It is obvious on this curve that a culture with a certain initial ratio has reached a higher stage of fermentation at the time of comparing the cultures than other cultures which received slightly more of the *B. subtilis* dilution.<sup>1</sup> Several explanations of this are possible, but the most logical seems to be from a mathematical comparison of the curves. A higher ratio of *B. subtilis* to *Strept. lacticus* may stimulate the first stages of fermentation sufficiently to stop the increase of *B. subtilis* before it reaches a concentration reached in a culture which had originally fewer *B. subtilis* organisms, since the latter has more time to develop (graph 2). Thus the "optimum" ratio would be that which influenced the fermentation up to the point beyond which the *B. subtilis* will not increase the least, and yet there attained the highest concentration. This concentration would be higher than in those cultures receiving more *B. subtilis* inoculum, and should hence in the long run give more stimulus. The "optimum" ratio represented in this curve (graph 3) was: *B. subtilis*/*Strept. lacticus* = 22/10,000.

Finally comparisons were made with clarified and unclarified associative cultures, presuming the larger organisms to be thrown out more than the smaller, and thus influencing the fermentation by disturbing the associative ratio of the two organisms. The

<sup>1</sup> It is necessary to keep in mind that the rate of fermentation can be stimulated only between the ordinary rate of multiplication of *Strept. lacticus* and its maximum physiological rate. Hence at room and lower temperatures under the optimum temperature for *Strept. lacticus* it is capable of greater stimulation than when warm. This has been demonstrated experimentally.



Simultaneous Ratio Effects at Two Different Stages

Table No. I

Ratio s/l	Acidity
Lact.	20.0
1	27.5
4	25.0
7	26.0
10	24.5
13	24.0
16	24.0
19	23.0
22	23.0
25	24.0
28	22.0
31	23.0
34	22.5
37	23.5
40	23.0

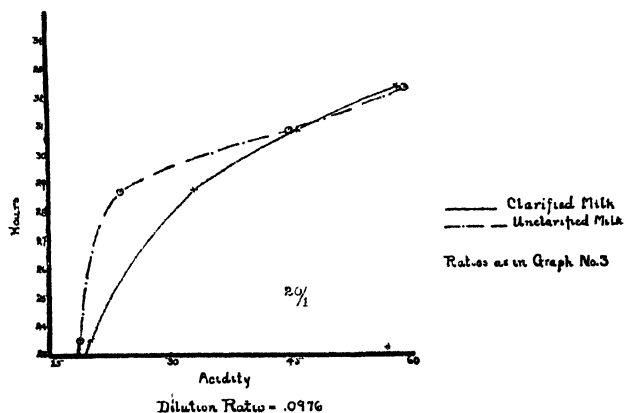
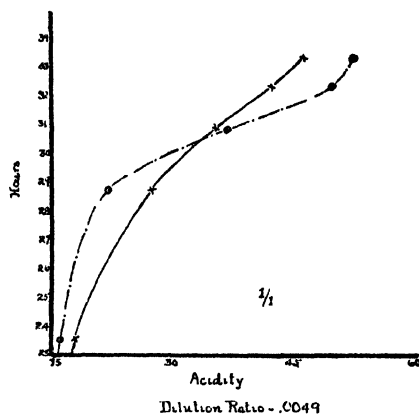
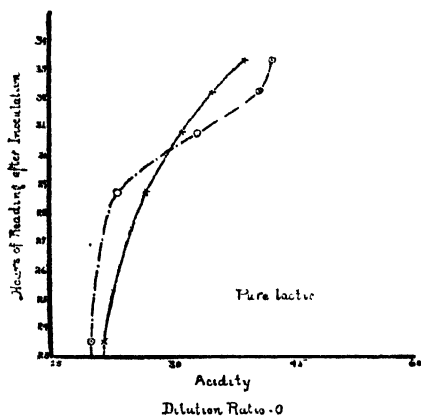
Table No. II (plotted)

Ratio s/l	Acidity
Lact.	27.0
1	47.0
4	50.0
7	45.5
10	45.5
13	46.0
16	40.5
19	38.5
22	39.0
25	37.0
28	38.0
31	37.5
34	39.0
37	37.5
40	41.0

Acidity or ordinate readings of curve correspond to acidity readings in table

Dilution drops corresponding to s in ratio of tables furnish the abscissa readings

The points along curve represent the acidity of the different cultures read at a given moment. Graph No. 3



Showing Comparison of Clarified and Unclarified Milk  
in Associative Cultures

Graph No. 4

results allow of considerable speculation, because so many influences other than association are present and intimately bound together with the introduction of clarification that quantitative conclusions on the associative basis are unwarranted. All that may be safely said is that clarification stimulates lactic fermentation in its early stages, be it with pure or associative culture, sufficiently so that whatever unbalancing of the ratio of the two organisms may take place makes no great apparent difference. Since the clarified cultures in the later stages of fermentation are behind the unclarified in the pure control of *Strept. lacticus* we can not draw any warranted conclusions from the associative cultures showing the same thing. That the associative influence is present in clarified milk is clear; if clarification influences association, however, its connection with these studies of the two organisms is not apparent.

It may be concluded that: (1) the presence of *B. subtilis* in milk influences lactic fermentation; (2) the higher the concentration of *B. subtilis* in the original milk the greater the stimulus in the earlier stages of fermentation; (3) the "optimum" ratio of *B. subtilis* to *Strept. lacticus* in the original milk which will give the greater stimulus in the later stages of fermentation is very small—approximately 22 to 10,000; (4) the stimulus is more evident at low temperatures than at high; (5) association plays an undeterminable part in the differences in lactic fermentation caused by clarification.

#### REFERENCES

- (1) BUCHNER: München Arztl. Intelligenzbl., No. 50, 1885.  
CARNOT: Comptes Med. Soc. de Biol., p. 765, 1898.  
DUCLAUX: Traité de Microbiologie, Paris, I and III, 1898-1900.  
GARRÉ: Korrespondenzol. f. Schweiz. Aerzte., Bd. 17, 1887.  
MARSHALL, C. E.: Bulletins 23, 29, 33, Mich. Agr. Col., 1905; Science, February 26, 1915.  
THIBAUT: Cent. f. Bakt., Ab. II, Bd. 9, S. 743, 1902.  
VAUGHAN: Infection and Immunity, Chap. III.  
WARD: Philos. Trans., vol. 50, 1892.

# THE USE OF FERMENTED MILK AND MILK DIETS IN CONTROLLING INTESTINAL PUTREFACTION

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The use of fermented milks and milk diets in controlling intestinal disorders has long been recognized. Especially the use of buttermilk and milk soured by *Bacillus bulgaricus* has been recommended by physicians. Earlier investigators have generally ascribed the beneficial action of these sour milks to the antagonistic action of *Bacillus lacticus* and *Bacillus bulgaricus*. The lactic acid developed by these organisms was considered as the controlling factor in restraining the development of intestinal putrefactive organisms.

According to Metchnikoff (1) *Bacillus bulgaricus* can be implanted in the intestine and has the power to destroy harmful bacteria, such as cause putrefaction of the intestinal contents.

Leava (2) a German investigator, carried on extensive experiments upon himself. He also claims that *Bacillus bulgaricus* can be implanted in the intestine by ingesting large quantities of the organism grown in milk. *Bacillus bulgaricus*, according to his report, was recovered from the feces after the fifth day, and at the same time the amounts of aromatic oxy-acids, and hippuric acid and conjugated acids in the urine decreased considerably.

Belonovsky (3) experimented upon mice, feeding them sterilized wheat and cultures of *Bacillus bulgaricus*. After twelve days the character of the intestinal flora showed a marked change from Gram-negative to Gram-positive organisms and a decrease in gas producers. Examination of the feces however failed to show any predominance of *Bacillus bulgaricus*. He believes that the beneficial action is not due altogether to the *Bacillus bulgaricus* or the lactic acid, but that other products play an important part also.

Cohendy (4) carried on extensive experiments on thirty hospital patients with the use of milk soured by *Bacillus bulgaricus*. His results also show a marked change in the character of the intestinal flora. This change was accomplished by the deodorization of the feces and a decrease in the quantities of conjugate sulphates in the urine. His conclusion is that the beneficial results are due to the *Bacillus bulgaricus*.

Sinclair (5) experimented with milk soured by *Bacillus bulgaricus* on over a hundred children suffering from infant enteritis and ileocolitis. He also reported a rapid improvement in practically all cases.

Hundreds of other cases could be mentioned proving the value of milk soured by *Bacillus bulgaricus* in the treatment of intestinal disorders. As stated before, earlier investigators generally ascribed this property to the *Bacillus bulgaricus*. Recent experimentors, however, seriously question this as the true explanation and attach much more importance to the influence of the diet.

Dr. Rettger (6) of Yale University conducted extensive experiments with young chicks. As in previous cases mentioned, the favorable influence of the milk feeding was very striking. Mortality was frequently reduced to at least one half of that amongst chicks receiving no milk. Milk-fed chicks frequently gained twice as much in weight as those not receiving milk. Chicks artificially infected with *Bacterium pullorum* (bacteria causing white diarrhea in chicks) showed a much greater resistance when milk fed.

However, the same results were obtained whether *Bulgaricus* milk or whole sweet milk was fed. His conclusions therefore were that the unique properties of this food exist in milk as such rather than in milk acids or sour milk bacteria that may be present.

Dr. Herter (7) has shown that the products of intestinal decomposition in normal nurslings and bottle-fed babies are remarkably small in amount, when the large number of bacteria inhabiting the lower part of the intestinal tract is considered. He also brings out the fact that the intestinal flora in these cases is a very simplified one. Instead of the varied mixed flora

of the adult, only a few types are predominant. In breast-fed babies *Bacillus bifidus* and *Bacillus acidophilus* are predominant. In bottle-fed babies there is a slight increase in the *Bacillus coli* group and a decrease in the bifidus type, while *Bacillus acidophilus* predominates.

The great abundance of *Bacillus bifidus* and *Bacillus acidophilus* in the simple flora of the healthy nursling or bottle-fed baby should be of considerable interest to the investigator. Especially so when compared with the varied mixed flora of the adult in which *Bacillus coli* commonly predominates. While *Bacillus coli* itself is considered a normal inhabitant of the adult intestinal tract, we usually find associated with it a host of other organisms the combination of which produces various degrees of intestinal putrefaction and its resulting disorders and diseases.

*Bacillus bifidus* and *Bacillus acidophilus* on the other hand show a strong antagonistic action against the coli type of organisms. They are carbohydrate consuming organisms and slow to attack proteins having practically no proteolytic action. Under favorable conditions they rapidly eliminate the *Bacillus coli* and its allies. Such favorable conditions seemingly exist in the case of nurslings or bottle-fed babies where we have a minimum of intestinal putrefaction, with a simple flora of the acidophilus and bifidus type.

These facts suggested to the writer that a simplified intestinal flora with *Bacillus acidophilus* and *Bacillus bifidus* predominating is associated with a minimum degree of intestinal putrefaction. Further that a milk diet of some kind is essential in bringing about this simplified flora.

It was with these ideas in mind that experiments were started to shed more light on this important question and to determine first, whether or not the simplified flora found in nurslings or bottle-fed babies can be brought about in the adult human being and second whether the organisms themselves are the causative factors or whether their abundance is due to the influence of certain diets furnishing a preferential medium for their development.

To study the effect of various milk diets on the intestinal flora and the amounts of decomposition products produced, six individuals placed themselves on certain milk diets for 28 days. The kind of diets used were:

1. Milk inoculated with *Bacillus bulgaricus*, *Bacillus lacticus* and *Bacillus acidophilus*.
2. Normal sweet whole milk of grade A quality.
3. Watery suspensions of *Bacillus acidophilus*, *Bacillus bulgaricus* and *Bacillus bifidus*.

From 2 to 4 quarts of the various milks were consumed and in addition a regular diet of meat, cereals and vegetables. As a natural consequence of consuming from two to four quarts of milk the quantity of the other foods was very materially reduced.

Observations were made on the nature of the intestinal flora and the production of the decomposition products as indicated by tests for phenol, indol, skatol and indican in the feces and urine. The procedure was to isolate the organisms from the feces by preparing a ten per cent suspension of one part of fecal material in nine parts of physiological salt solution. This suspension was then used for further study as to:

1. Plating on aerobic and anaerobic plates.
2. Staining or smears to study staining properties.

The Gram positive stain was found very helpful because the *Bacillus coli* group is gram negative, while *Bacillus bifidus*, *Bacillus acidophilus* and *Bacillus bulgaricus* are gram positive.

3. Inoculation of fermentation tubes for gas production.
4. Cultural characteristics in milk.
5. Bio-chemical study for decomposition products as phenol, skatol, indol and indican.

Records were kept as to the movement of the bowels and general welfare of the patient as indicated by gain or loss in weight. More detailed description of methods are reported in Bulletin 104 Storrs Experiment Station (8).



## EFFECT OF FERMENTED MILK IN THE DIET

*Bacillus bulgaricus* is a rather common inhabitant of milk and as such appears as long slender overlapping threads. It is Gram positive and with methylene blue frequently shows granules in the cell. Its optimum growing temperature is 100°F. and in milk it forms a homogeneous curd coagulating the milk usually in six to twelve hours. Action on dextrose, lactose and sucrose broth is characteristic by formation of acid without gas.

Whole milk of grade A quality was pasteurized by heating to 145°F. and holding for thirty minutes. Half of the milk was then cooled to 100°F. and inoculated with a pure culture of *Bacillus bulgaricus*, while the other half was inoculated with a pure culture of *Bacillus lacticus* at 70°F. After about 18 hours of incubation at the respective temperatures the milk was again put together in a small hand churn and churned for about ten minutes. The milk was then cooled, bottled and stored in a refrigerator till used. The addition of *Bacillus lacticus* cultures moderates the acidity and takes away the sharp, puckering taste of a pure *bulgaricus* culture. The fermented milk thus produced had an approximate acidity of 1.10 per cent.

Five out of six completed the experiment, practically all of whom showed an increase in weight and improvement in physical condition. One of the patients was forced to discontinue the fermented milk due to stomach trouble. At the beginning of the experiment two of the patients selected suffered slightly from indigestion, while all suffered more or less from constipation. All reported improvement in the movement of the bowels. Examination of the feces and urine showed a marked decrease in putrefactive decomposition products. The relief from constipation may have been an important factor in causing a decrease in the decomposition products. Table 1 is a summary of the results.

The intestinal flora in all cases changed from a predominance of Gram negative to a flora which showed a large number of Gram positive organisms. Attempts were made to recover the *Bacillus bulgaricus* from the feces. The results were unsuccessful.

ful in practically all cases. Several times Gram positive organisms resembling *Bacillus bulgaricus* were isolated. These organisms answer the description of *Bacillus acidophilus* of Moro. Under the microscope they appear as long slender bacilli which

TABLE 1  
*Effect of fermented milk in the diet*

PATIENT	INTESTINAL FLORA		DECOMPOSITION PRODUCTS		CONSTIPATION		WEIGHT	
	Before	After	Before	After	Before	After	Before	After
1	+--	++-	++	+	++	--	165	168
2	+--	+++	+++	++-	+++	--	119	124
3	+--	++-	++	--	+	--	133	134
4	+--	+++	++++	+-	++++	---	141	150
5	+--	++-	++	+-	+++	+++	130	129½
6	+--	++-	+++	++-	+	-	154	154

NOTE: Gram negative is indicated by -- which shows that the type of organism is of the coli or putrefactive type.

Gram positive is indicated by + which shows that the type of organism is of the acidophilus or beneficial type.

Patient no. 5 discontinued experiment after five days.

TABLE 2  
*Formation of acid in milk by Bacillus acidophilus*

INOCULATION	INITIAL ACIDITY IN MILK	AFTER 24 HOURS	AFTER 48 HOURS	AFTER 72 HOURS
First.....	0.15	0.43	0.60	0.65
Second. . . . .	0.17	0.49	0.61	0.69
Third. . . . .	0.16	0.63	0.80	0.81
Fourth.....	0.21	0.53	0.77	0.85
Fifth.....	0.15	0.75	0.87	0.93
Sixth.....	0.15	0.75	0.97	1.09
Seventh.....	0.17	0.83	1.01	1.19
Eighth.....	0.16	0.90	1.34	1.58

NOTE: These results are an average of six different strains. The highest reached an acidity of 1.97 per cent in seventy-two hours.

show a Gram positive stain. Colonies on plain or whey agar are microscopical in size and resemble colonies typical of *Bacillus tetani*. Action on dextrose, lactose and sucrose broth were characteristic by formation of acid and no gas. They are very

similar to *Bacillus bulgaricus*, but did not form acid as rapidly nor is the maximum acidity as high. On propagation in milk cultures they may however develop a considerable acidity as brought out in table 2.

The principal point of difference is that *Bacillus acidophilus* can readily be recovered from the feces while *Bacillus bulgaricus* cannot.

When grown in milk *Bacillus acidophilus* forms a smooth homogenous curd which has a pleasing mild acid flavor. As such it makes a very palatable milk drink and seems much better adapted for use in the preparation of a fermented milk than *Bacillus bulgaricus*. The *acidophilus* milk produces the characteristic changes of intestinal flora and decomposition products even more strikingly than the *bulgaricus* milk and *Bacillus acidophilus* could be isolated from the feces in from five to eight days.

#### USE OF SWEET WHOLE MILK IN THE DIET

The procedure of the experiment was the same as where fermented milks were used. All tests and observations were made under as nearly identical conditions as was possible to

TABLE 3  
*Effect of sweet whole milk in the diet*

PATIENT	INTESTINAL FLORA		DECOMPOSITION PRODUCTS		CONSTIPATION		WEIGHT	
	Before	After	Before	After	Before	After	Before	After
1	+--	++-	++	+-	++	+-	166½	167½
2	+--	++-	+++	++-	+++	++-	121	127½
3	+--	+++	+++	+++	+-	++	133	133½
4	+-	++-	+++	++-	+++	+++	147	146
5	+--	+++	++	--	--	--	134	139
6	+--	++-	++++	++-	+-	--	153½	155½

For explanation see note under table 1.

attain. This time, however, sweet whole milk meeting certified requirements was used. Six patients were fed for a period of twenty-eight days on a regular diet of meats, cereals and vegetables in reduced quantities to allow for the additional food furnished by the daily use of 2 to 4 quarts of milk.

The intestinal flora showed the same marked change as when feeding the fermented milks. It took from eight to ten days before the slides showed considerable numbers of Gram positive organisms of the bifidus and acidophilus types. There was a decrease in the decomposition products. However, patients suffering from constipation were not relieved as they were on the bulgaricus diet. Results are shown in table 3.

Clinical symptoms and general health improved and there was a gain in weight with the exception of two patients who reported severe constipation as a result of the milk diet. Others, however, reported a laxative effect.

#### USE OF BACILLUS ACIDOPHILUS, BACILLUS BULGARICUS AND BACILLUS BIFIDUS WITHOUT MILK IN THE DIET

Pure cultures of *Bacillus acidophilus*, *Bacillus bulgaricus* and *Bacillus bifidus* were grown on dextrose agar. Watery suspensions of the bacilli were then made from these dextrose cultures.

TABLE 4

*Effect of watery suspensions of Bacillus bulgaricus, Bacillus acidophilus and Bacillus bifidus*

PATIENT	INTESTINAL FLORA		DECOMPOSITION PRODUCTS		CONSTIPATION		WEIGHT	
	Before	After	Before	After	Before	After	Before	After
1	+-	++	++	++	+-	+-	144	144½
2	+-	+-	+-	+-	+-	+-	151	153
3	+-	+-	++	+-	--	--	152	151½
4	+-	+-	--	+-	--	--	121	120
5	+-	++	--	--	+-	+-	134	136
6	+-	+-	+-	+-	+-	+-	146	148

For explanation of table see note under table 1.

These watery suspensions were mixed with the food and thus ingested. Six patients followed a regular diet of meat, cereals and vegetables, but no milk was used during four weeks of the experiment. All other conditions were the same as when fermented and sweet whole milk was fed.

No appreciable change could be noticed in the amount of decomposition products produced. The smears showed some

Gram positive bacteria, but large numbers of Gram negative organisms were present throughout the experiment. *Bacillus acidophilus*, *Bacillus bulgaricus* and *Bacillus bifidus* supplied to the diet in the form of watery suspensions seemingly affect the intestinal flora only temporarily and then only to a very slight extent. They do not cause any marked decrease in the production of intestinal decomposition products and their implantation without milk in the intestinal tract is very doubtful. The persons on test did not experience any favorable or unfavorable results from the ingestion of the large numbers of the organisms. Results are indicated in table 4.

#### GENERAL DISCUSSION AND CONCLUSION

One of the most striking facts to be noticed in the series of experiments is the characteristic change in the intestinal flora coupled with decreased intestinal putrefaction whenever milk constituted a large part of the diet. In these cases the intestinal flora changed from the usually Gram negative coli type to one in which Gram positive aciduric *Bacillus acidophilus* was prominent. In all instances where milk made up the principal part of the diet there was a marked decrease in the decomposition products.

The results from the use of *Bacillus bulgaricus* milk in connection with the regular diet was very gratifying. Intestinal putrefaction decreased markedly when at least two quart of the bulgaricus milk was used and the other food reduced proportionately. There was a general gain in weight, and those suffering from constipation were very much relieved. Of special interest is the change in the intestinal flora. Notwithstanding the fact that very large numbers of *Bacillus bulgaricus* were ingested with the milk, only a few could be recovered from the feces. *Bacillus bulgaricus* is therefore not a natural inhabitant of the human intestinal tract. The very closely related *Bacillus acidophilus* could be found in large numbers, but only after about eight or ten days use of the bulgaricus milk. When milk was discontinued the *Bacillus acidophilus* gradually disappeared

and the flora again showed a large number of Gram negative organisms.

The same held true with the whole sweet milk experiment, which gave practically as good results as bulgaricus milk. This fact becomes still more significant when it is considered that *Bacillus acidophilus* became a common inhabitant of the intestinal tract without the ingestion of the organism itself. This would indicate that *Bacillus acidophilus* is naturally an intestinal organism, and that its presence or absence is dependent upon the nature of the food consumed. The diet or nature of the food then is the controlling factor. The character of the intestinal flora is dependent on, and alters with the type of food. The ingestion then of large numbers of certain bacteria in tablet or liquid form without the proper diet can be of little permanent value. This fact was brought out by the experiment in which watery suspensions of *Bacillus bulgaricus*, *Bacillus acidophilus* and *Bacillus bifidus* were consumed. The intestinal flora throughout the experiment showed large numbers of Gram negative types, and there was no appreciable decrease in the decomposition products.

The beneficial effect of milk in the successful treatment of various intestinal diseases as typhoid fever has for sometime been known to the medical profession, but not until recently has it been realized that the explanation lay in its ability to change the intestinal flora from the putrefactive type to the Gram-positive aciduric *Bacillus acidophilus* group, the presence of which is associated with a minimum amount of putrefaction and the general well-being of the individual. Whether fermented milk or fresh sweet milk is preferable depends upon conditions. If the subject is constipated the fermented milk may prove very helpful. On the other hand, some individuals apparently do not tolerate acid milk, and in such cases the sweet milk would be best. Approximately three to four quarts should be used daily, in half pint quantities taken every hour or two. Other foods should be reduced proportionately, and in certain cases it may be advisable to leave them out entirely and increase the milk to 4 or even 6 quarts, depending on the needs of the patient.

There may be conditions when it is advisable to have in the intestine at once large numbers of the *Bacillus acidophilus*. A fermented milk prepared with *Bacillus acidophilus* would be admirably suited for such needs. Instead of using the bulgaricus culture an acidophilus culture should be used. This provides the proper diet in the form of milk and large numbers of the desirable organism which is a natural inhabitant of the intestinal tract. The acidophilus milk has a pleasing mild acid flavor and a rich creamy consistency.

It seems to the writer that such a fermented milk offers great possibilities in the treatment of various intestinal disorders. As yet much work remains to be done especially in preparing a commercial product which can readily be manufactured in the average milk plant.

Experiments covering this phase of the work are now under way in the Dairy Laboratories of the Connecticut State College, at Storrs.

So far the work has brought out the importance of the diet in controlling intestinal putrefaction and disorders. It was shown that a change in the food alters the nature of the intestinal flora, and that milk is the one food which can bring about so characteristic and beneficial a change in the intestinal flora from the putrefactive to the acidophilus type. Already the medical profession is using Nature's Own Food, milk, to combat various diseases, and will continue so even more in the future, as the importance of the proper food in prevention and treatment of disease is realized.

#### REFERENCES

- (1) METCHNIKOFF, E.: Prolongation of Life.
- (2) LEAVA, J.: Berl. klin. Wch. Chr., 1908, xiv, 922.
- (3) BELONOVSKY, J.: Ann. De L' Inst.
- (4) COHENDY, M.: Compt. Rend. Soc. de biol., 1906, lviii, 602.
- (5) SINCLAIR: Medical Record, 1913.
- (6) RETTGER, LEO F.: Journal of Experimental Medicine, xxi, 1915, 365.
- (7) HERTER, C. A.: Common Bacterial Infections of the Digestive Tract.
- (8) Storrs Experiment Station Bulletin 104, Storrs, Connecticut.

# A COMPARISON OF THE DECANTATION METHOD WITH OTHER METHODS FOR THE DETER- MINATION OF FAT IN BUTTER

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Many methods which may be used in the laboratory for the determination of the percentage of fat in butter have been devised. None of these methods, however, is directly applicable in creamery control analyses, because of the time involved and the skill necessary in their manipulation. Certain of these methods have been modified in such a manner as to decrease the time and the skill required to obtain a determination of sufficient accuracy for creamery work.

An attempt has been made in this work to compare one of the newer of these modified methods, the Kohman<sup>1</sup> decantation method, with the Mojonnier method and with two of the slower laboratory methods, the A. O. A. C. and the Roesse-Gottlieb. In the last three of these methods all weighing is done with an analytical balance.

In the decantation method the moisture was determined in a 10-gram sample, using an aluminum cup with a pour-out lip. The cup was about 2 inches inside diameter and  $2\frac{1}{4}$  inches deep. A Torsion moisture balance—style 1700—was used for all weighing done in both the moisture and the fat determinations. Any other type of moisture balance of equal sensibility could be used just as successfully.

After the completion of the moisture determination, about 50 cc. of petroleum ether was poured into the cup and was thoroughly mixed with the dry butter. The fat dissolved in the ether while the curd and salt, which are insoluble, settled to the bottom of the cup. When the sediment had completely separated (usually not more than two or three minutes are necessary), the ether solution of the fat was poured off, care being taken not

<sup>1</sup> E. F. Kohman, J. Ind. Eng. Chem., xi, 36, 1919.



to lose any of the sediment with the ether. About 30 cc. of the solvent was added to the sediment in the cup. The mixture was stirred, allowed to settle, and the solvent was poured off. In each case the solvent should be poured off as completely as is possible without loss of sediment. The cup with its contents was then dried on a water bath or hot plate. The drying may be done directly over a very small non-luminous flame if care is taken to avoid ignition of the remaining traces of petroleum ether. After the ether had completely evaporated, the cup was cooled and the loss in weight due to the extraction of the fat was determined. A set of weights ranging from 0.01 gram to 5 grams is necessary for this weighing. The moisture weights during the fat determination must be left in exactly the same position as they were placed to show the percentage of moisture. The loss in weight of the cup and contents represents the weight of fat in 10 grams of butter. This weight multiplied by 10 gives directly the percentage of fat in the sample.

The percentage of salt may be determined by applying to the contents of the cup after the completion of the fat determination any of the salt tests based upon a 10-gram sample of butter.

The percentage of curd may be found by difference:  $100 - (\text{percentage moisture} + \text{percentage fat} + \text{percentage salt}) = \text{percentage curd}$ .

In table 1 duplicate results obtained in the analysis of eight different samples of butter by each of the four methods named above are listed. In no case does the amount of fat determined by the decantation method vary from the determination by any of the other three methods by as much as 0.2 per cent, and in most cases the difference is less than 0.1 per cent.

As a further verification of the accuracy of the decantation method the ether solution of the fat was poured directly from the cup into a weighed Erlenmeyer flask. The ether was evaporated on an electric hot plate and the flask and fat dried to constant weight at 100°C. in an electric oven. The results of these determinations are listed in table 2. The analyses numbered 1 to 8 are the results obtained from the samples correspondingly numbered in table 1. Analyses 9 to 12 are the results

of samples tested only by the decantation method and by the direct weighing of the fat recovered from the decanted solution.

In 15 of the 24 determinations listed the difference between the two methods is less than 0.1 per cent and in none of the other 9 does it reach as much as 0.2 per cent.

TABLE 1

*Comparison of the decantation method with the Mojonnier, Roese-Gottlieb, and A. O. A. C. methods*

ANALYSIS NUMBER	A. O. A. C.		ROESE- GOTTLIEB	MOJONNIER	DECANTATION	
	Water	* Fat	Fat	Fat	Water	Fat
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	14.29	82.35	82.40	82.41	14.2	82.3
	14.32	82.34	82.37	82.40	14.3	82.3
2	14.08	82.46	82.52	82.46	14.0	82.5
	13.93	82.43	82.49	82.42	14.0	82.4
3	14.17	82.17	82.21	82.19	14.2	82.3
	14.20	82.11	82.20	82.19	14.1	82.2
4	14.66	81.90	81.93	81.93	14.5	81.9
	14.53	81.87	81.89	81.90	14.6	81.8
5	13.69	82.23	82.25	82.29	13.8	82.2
	13.54	82.19	82.24	82.24	13.7	82.2
6	14.95	80.45	80.44	80.35	15.1	80.5
	15.07	80.38	80.36	80.41	15.1	80.3
7	13.81	82.05	82.13	82.05	13.9	82.0
	13.87	82.08	82.09	82.06	13.9	82.0
8	14.54	81.20	81.11	81.14	14.5	81.2
	14.74	81.06	81.15	81.17	14.5	81.1

The results given in the tables show very distinctly the accuracy of the decantation method as compared with other methods used in the analysis of butter for fat.

The decantation method as applied to creamery control work in butter making has the following advantages:

1. The method is accurate, giving results which check very closely with the slower laboratory methods.

2. The method is simple and easy to manipulate. No more skill is necessary for its successful use than is required in the usual moisture determination.

TABLE 2

*Comparison of the decantation method with the method of direct weighing*

ANALYSIS NUMBER	DECANTATION METHOD, FAT	DIRECT WEIGHING, FAT	DIFFERENCE
	<i>per cent</i>	<i>per cent</i>	
1	82.3	82.38	0.08
	82.3	82.32	0.02
2	82.5	82.60	0.10
	82.4	82.58	0.07
3	82.3	82.34	0.04
	82.2	82.28	0.08
4	81.9	82.04	0.14
	81.8	81.92	0.12
5	82.2	82.31	0.11
	82.2	82.24	0.04
6	80.5	80.59	0.09
	80.3	80.41	0.11
7	82.0	82.01	0.01
	82.0	82.14	0.14
8	81.2	81.23	0.03
	81.1	81.25	0.15
9	82.7	82.63	-0.07
	82.7	82.68	-0.02
10	80.75	80.94	0.19
	80.8	80.93	0.13
11	81.2	81.21	0.01
	81.2	81.16	-0.04
12	82.3	82.24	-0.06
	82.3	82.32	0.02

3. Not more than fifteen to twenty minutes are required for the determination of both moisture and fat. A few minutes more will permit the determination of salt and curd if desired.

4. The only apparatus and material necessary in addition to that used in the ordinary moisture test is: (a) a set of weights ranging from 0.01 gram to 5 grams; and (b) a supply of petroleum ether with boiling point 30° to 50°C. or 50° to 60°C. (The expensive fat-free petroleum ether is not necessary.)

# ABSTRACTS AND REVIEWS OF DAIRY LITERATURE

## MICROBIOLOGY AND MICROANALYSIS OF FOODS<sup>1</sup>

"This volume is intended as a guide to the study of the microbiological decomposition changes in foods. It also presents a practical working basis for ascertaining the decomposition limits of foods suitable for human consumption, by means of the direct methods of micro-analysis."

The text of 260 pages is addressed to army dietitians and food examiners and in keeping with the limitations surrounding war conditions attention is directed almost exclusively toward information obtainable through the microscope. To those accustomed to elaborate laboratory equipment the character and variety of the information so obtainable is quite a revelation. Although we are no longer at war there is still need of methods for a rapid and accurate examination of foods and in this volume, which covers practically all kinds of foods, such methods are well arranged and well indexed.

While the question of methods has been well handled there will undoubtedly be much difference of opinion regarding the deductions drawn from examinations of food. His statement that "Grade 'A' and Grade 'B' milks are of equal value to the army" will shock some people. His conclusion that cheeses should not be used as an army food because of the liability of their undergoing secondary toxic decomposition will likewise appear strange to others. His evident suspicion of canned goods is in sharp contrast with the results of the study by Rosenau and his associates.

As a matter of fact, wholesomeness of foods has long been a favorite field for fable and fancy. It is asking too much that the author should divest himself of all the myths of his childhood and confine himself to prosaic facts. Filth, rotting bacteria, and ptomaine poisonings are very real imps to the author and to a majority of the public.

While at present it is frankly impossible to harmonize the extreme views held by many regarding the relations of germ life to wholesomeness of food this author should be credited with an evident desire to take a moderately conservative position in such matters. The book is a valuable contribution and will repay careful examination by those interested in the subject.

H. A. HARDING.

<sup>1</sup> By A. Schneider,  
P. Blakeston's Son & Co.

## DAIRY NOTES

J. W. HENDRICKSON

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### ARKANSAS

Prof. H. E. Dvoradhek of the dairy department, University of Arkansas, reports some investigations in progress in the use of legume crops as silage for dairy cattle. Cow peas and soy beans are used in combination with corn and cane. A progress circular or bulletin may be published soon. The Arkansas station is also doing some research work on the cost of raising dairy cattle.

### CALIFORNIA

Among the recent publications from the California University Dairy department is a circular on the subject "The Babcock Test for Butterfat in Milk, Cream and Skimmed Milk," by J. C. Marquardt and a bulletin on "The Use of White Fir and Choice Lumber in the Manufacturing of Boxes Used for Shipping and Storing Butter," by Prof. S. D. Turnbow who scored the butter when it was placed in the boxes and will also make examination at frequent intervals. In California there has been a prejudice against the use of boxes made from any other than spruce lumber. It is believed that there are other woods that are cheaper and that will give just as satisfactory results as the more expensive spruce boxes.

Assistant Professor S. D. Turnbow became a member of the staff of the California Dairy Industry during the present year.

Mr. H S. Beard formerly assistant professor of dairy industry has accepted a position with the Northern California Milk Producers Association.

Mr. S. L. Denning also formerly of the California Dairy Industry Department is now with the sales department of the Premier Machinery Company of San Francisco.

### CONNECTICUT

Prof. R. C. Fisher of the Connecticut Agricultural College has a new publication on "The Use of Fermented Milk and Milk Diets to Control Intestinal Putrefaction."

On February 1, 1920, Mr. Wm. A. Rhea, B.S., University of Missouri, M.S. from Cornell University, resigned from his position at the Connecticut Agricultural College as extension dairyman and is now with a banking institution at Pierce City, Missouri. The vacancy was filled by the selection of Mr. P. A. Campbell, a graduate from the New Hampshire State University and formerly professor of animal husbandry at the University of Maine. During the last ten years Professor Campbell has been connected with two of the largest breeding establishments in the East, namely Balsam Stock Farm, Dixville Notch, New Hampshire, and Ayrdale Stock Farm, Bangor, Maine.

## IDAHO

Mr. H. A. Bendixen, M.S. of the Iowa State College, has accepted the position of assistant professor of dairy husbandry in the University of Idaho to succeed Mr. E. F. Sass. Mr. Bendixen's work will be along the line of milk testing, butter making, cheese making and ice-cream manufacturing—and will have charge of the teaching and research work along these lines.

## ILLINOIS

Professors H. A. Harding and M. J. Prucha, of the Illinois Station, have just issued a bulletin No. 228 on the subject "An Epidemic of Ropy Milk," in which they state that ropy milk is due to the growth of an organism resulting in increased viscosity of the milk. While no evidence has been produced to indicate that the healthfulness or the flavor of the milk have been changed, the consuming public does not look with favor upon this class of product. In the epidemics studied, sanitary methods applied to utensils, milk house equipment and to cows and stable surroundings as well, seem necessary in order to effect complete eradication of the trouble.

## IOWA

Prof. B. W. Hammer of the Iowa State College has quite recently issued several general and research bulletins, among which are "Bacteriological Results Obtained in Practice with Vat Pasteurization and with One of the Final Package Methods," also, "A Bacteriological Study of the Methods of Pasteurizing and Homogenizing the Ice Cream Mix." The research bulletins are on the subjects "Studies on

Abnormal Evaporated Milk," "Studies on Formation of Gas in Sweetened Condensed Milk," and "The Volatile Acid Production of Starters and of Organisms Isolated from Them."

In first research bulletin mentioned "An abnormal condition in evaporated milk, evident in the original milk as a bitter flavor and abnormal odor, was studied and found to be due to an organism that it is believed has not been previously described and for which the name *Bacillus amarus* is proposed."

In the second research bulletin the gas formation in sweetened condensed milk was found to be due to a budding organism. Professor Hammer states that "Since the yeast can grow in a saturated solution it is possible that the milk solids play a part in keeping down growth."

In the study of starters, Professor Hammer assisted by Mr. D. E. Bailey came to the conclusion that more than one organism is often present in starters of satisfactory quality. They also state that the high volatile acidity of starters is not due to the action of *B. Lactis acidi* alone.

Mr. E. W. Renner, instructor in market milk at the Iowa State College is now with the Decatur Ice Cream Company, Decatur, Illinois.

Mr. S. J. Pearse, formerly a Nebraska man with M.S. degree from Iowa State College has taken the place of Mr. Renner.

Mr. J. F. Jarvies, instructor in ice cream making, left to go with the Douglas Ice Cream Company, of Chariton, Iowa. Mr. R. L. Neasham recently with the Flynn Dairy Company of Des Moines has been selected to fill the vacancy.

#### KANSAS

Prof. N. E. Olsen, formerly assistant professor of dairy husbandry, is now with the Columbia Dairy Products Company at Vancouver, Washington. Mr. F. W. Athenson in charge of advanced registry work has become field man for the Southwest Jersey Breeders' Cattle Club, with headquarters at Kansas City, Missouri.

#### MARYLAND

The following bulletins have just been issued by the Maryland Station: "Cooling of Milk and Cream on the Farm," Farmers' Bulletin No. 976, U. S. Dept. of Agri., J. A. Gamble; "The Straining of Milk on the Farm," by Ernest Kelly and J. A. Gamble, Farmers' Bulletin No. 1019; U. S. Dept. of Agri. Bulletin No. 744, "Cooling Milk and



Storing and Shipping It at Low Temperature," by J. A. Gamble and John T. Bowen, U. S. Dairy Division. A list of the breeders of pure-bred dairy cattle in Maryland (in process of publication) by Messrs. Meade and Gamble. The Babcock Testing of Milk by J. A. Gamble.

In coöperation with the Market Milk Section of the United States Dairy Division, a study on the effects of feeds on the flavors and odors of milk is being made.

E. H. Parfitt, graduate student in dairying, has left to take up work in the laboratories of the United States Dairy Division. A graduate assistant to take his place will be available July 1 and a teaching assistant in dairy husbandry, October 1.

R. H. Ruffner, formerly professor of animal and dairy husbandry at this institution, is now head of the animal and dairy husbandry department at the North Carolina A. & A. College, West Raleigh, North Carolina. Mr. A. C. Stanton, formerly his assistant, is in charge of the Seward Stock Farm, Petersburg, Virginia.

#### MINNESOTA

R. M. Washburn, who was for seven years professor of dairy husbandry in charge of dairy products in this institution, is now with the International Dry Milk Company, of Minneapolis, as technical expert. J. C. Cort resigned his University position during the year to assume the duties as manager of the Milford Meadows Stock Farm, Lake Mills, Wisconsin. E. O. Hansen, formerly assistant professor in this division, has severed his connection with this institution in order to become assistant manager of the Creamery Package Manufacturing Company, Minneapolis.

#### MISSISSIPPI

A publication on "Testing Milk and Its Products" has just been issued.

Mr. Earl Brentnall has been employed to do investigational work in dairy production.

#### MONTANA

Montana Station has issued a bulletin No. 131 on sunflower silage for dairy cattle, which should be of interest to those states that are not able to produce good corn silage.

## NEBRASKA

Mr. M. N. Lawritson, who has been in charge of the advanced registry testing work has become the dairy specialist in the extension department. Mr. Lawritson's place has been filled by the selection of Mr. J. W. Boehr, who received his M.S. degree in dairying at the University of Nebraska last year.

## NORTH DAKOTA

Professor J. R. Keithley who was formerly head of the department of dairy husbandry at North Dakota Agricultural College has gone to the University of Minnesota to take charge of dairy manufacturing.

## OKLAHOMA

The principal experimental projects carried on by the department of dairying includes the study of the grading of sour cream for the manufacture of butter during the hot summer months; factors in the commercial manufacture of ice cream which includes a study of the use of extra milk solids for improving the quality and increasing the overrun of ice cream; the commercial manufacture of cheddar cheese and the commercial pasteurization of milk in the final container.

Prof. C. A. Burns, assistant dairy-man, resigned in March to go into commercial work and the vacancy has been filled by the appointment of A. D. Burke of Columbus, Ohio, who comes to the department on July 1, 1920.

## PENNSYLVANIA

W. B. Combs, graduate at the University of Missouri, 1915, was placed in charge of the dairy manufacturing work in the Department of Dairy Husbandry, January 1, 1920, in the place of H. C. Yerger, Jr., who resigned to take up commercial work.

## SOUTH CAROLINA

South Carolina Dairy Division has the following experimental work under way: (a) Study to determine the most economical concentrate to supplement cotton seed meal as a feed for dairy cows in the south. This experiment is being undertaken in coöperation with the North Carolina and Alabama Stations. Various feeds are being compared with

velvet bean meal and wheat bran. (b) Comparison of corn silage and sorghum silage for milk production. (c) To ascertain the amount of feed necessary and cost of raising dairy calves to two years of age under South Carolina conditions.

#### SOUTH DAKOTA

Mr. H. A. Mathiesen who has served since September 1919 as graduate assistant in the dairy department of South Dakota State College resigned in March to become assistant field dairyman in Idaho.

#### CHANGES IN UNITED STATES DAIRY DIVISION PERSONNEL

W. E. Wintermeyer, assistant dairy manufacturing specialist, has resigned to accept a position with the Fisk Tire Rubber Company, at Raleigh, North Carolina.

Geo. J. Miller, dairy manufacturing specialist, engaged in creamery extension work in Mississippi, has resigned to accept a position as manager for the Watson and Avon Ice Cream Company, at Helena, Arkansas.

F. L. Brown, cooperative dairy extension man in New Hampshire, has resigned to become a county agent in New Jersey.

H. J. Childress, cooperative dairy extension worker in Oklahoma, has resigned to accept a position as county agent in the State of Kentucky.

Dr. N. R. Blatherwick, who has been engaged on problems in connection with the physiology of milk secretion, has resigned to become biochemist in charge of the chemical work with the Memorial Laboratory and Clinic, Santa Barbara, California, where he will conduct research work in metabolic diseases.

W. D. Wood, engaged in experiments to determine the food requirements for milk production in Louisiana, has resigned to accept a dairy extension position in South Carolina.

Clarence V. Castle, a graduate of the University of California and an instructor in animal husbandry and superintendent of the farm dairy at that institution for two years, has been appointed for the organization of cow-testing associations in the Western United States with headquarters at Salt Lake City, Utah.

Elliot H. Parfitt, of New York, has been appointed scientific assistant in dairying and assigned to cheese manufacturing investigations in the dairy research laboratories, taking the place of A. C. Weimar who was transferred to Grove City, Pennsylvania. Mr. Parfitt received his

degree of Associate in Agriculture from the Ontario Agricultural College (Guelph), 1916, and his B.S. in Agriculture from Toronto University in 1918. After graduating he was appointed as lecturer in dairy husbandry at Ontario Agricultural College, but enlisted, November, 1918, in the Medical Corps of the United States Army. In the fall of 1919 he was appointed instructor in dairy manufacturing at the Maryland Agricultural College.



# PROPORTIONING THE INGREDIENTS FOR ICE CREAM AND OTHER FROZEN PRODUCTS (THE BALANCE METHOD)

O. E. WILLIAMS

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Agriculture, Washington, D. C.*

One of the most satisfactory methods that can be used for proportioning the ingredients in making large ice cream mixes is what we have called "the balance method." It is a method that can be easily understood, is applicable to all combinations of ingredients and reduces to a minimum the chances of error in the calculations. Furthermore it furnishes an itemized record of the ingredients used for each mix. The proportions obtained by this method are based on five conditions:

1. The amount (pounds) of mix that will be necessary to produce the number of gallons of ice cream desired.
2. The composition (standard) of ice cream desired.
3. The amount of solid constituents necessary for the mix.
4. The quantity and physical condition of the ingredients on hand.
5. The composition of the ingredients to be used.

Five examples of this method of proportioning the ingredients are explained as follows:

## EXAMPLE I

*Mix.* Give the proportions for 350 gallons of ice cream testing approximately 14.5 per cent fat, 14 per cent sugar and 6.5 per cent milk solids not fat. The weight of the ice cream desired is 5 pounds per gallon.

*Stock on hand.* Sugar, 150 pounds of 28 per cent cream, 520 pounds of 43 per cent cream, and skim milk.

*Illustration*

TOTAL POUNDS DESIRED 1750	INGREDIENTS AND COMPO- SITION	COMPOSITION DESIRED		
		Fat 14.5 per cent	Sugar 14 per cent	m. s. n. f. 6.5 per cent
		Constituents necessary		
		253.75 pounds	245 pounds	113.6 pounds
		Constituents furnished		
<i>pounds</i>		<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
245.0	Sugar		245.0	
150.0	Cream, 28 per cent	42.0		10.0
492.5	Cream, 43 per cent	211.75		26.0
862.5	Skim milk, 9 per cent			77.6
1750.0		253.75	245.0	113.6

*How to determine the basic conditions*

*Condition 1.* To get the total number of pounds in the mix, multiply the desired number of gallons of ice cream by the number of pounds expected in one gallon of the finished product. For instance, in the first example 5 pounds is the desired weight of one gallon of ice cream, hence:

$$350 \times 5 = 1750 \text{ pounds of mix.}$$

*Condition 2.* The approximate composition of the ice cream desired in the first example is 14.5 per cent fat, 14 per cent sugar and 6.5 per cent milk solids not fat.

*Condition 3.* To find the amount of solid constituents necessary, multiply the pounds of mix by the percentage of fat, sugar and milk solids not fat as in the first example:

$$1750 \times 0.145 = 253.75 \text{ pounds of fat}$$

$$1750 \times 0.14 = 245.0 \text{ pounds of sugar}$$

$$1750 \times 0.065 = 113.7 \text{ pounds of milk solids not fat.}$$

*Conditions 4 and 5.**Quantity and composition report*

	COMPOSITION			QUANTITY ON HAND
	Fat	Sugar	m. s. n. f.	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
Cream.....	28		6.4	150 pounds
Cream.....	43		5.3	Plenty
Skim milk.....			9.0	Plenty

After these basic conditions are determined, write the pounds of mix, the percentage of constituents desired and the pounds of each constituent in table form and list the ingredients to be considered for the mix as shown in the illustrations.

*Particulars concerning example 1*

The calculations necessary in determining the proportions are as follows (consider the ingredients as they are listed):

*Sugar.* The amount of sugar is the same as the amount calculated for the mix since there is no cane sugar in the other ingredients.

*Cream (28 per cent).* The 150 pounds of 28 per cent cream does not contain more fat than is needed, hence the entire amount can be used.

*Cream (43 per cent).* The amount of 43 per cent cream can be determined by subtracting the amount of fat added by the 150 pounds of 28 per cent cream from the total amount required and dividing the remainder by 0.43, thus:

$$253.75 - 42 = 211.75$$

$$211.75 \div 0.43 = 492.5 \text{ pounds of 43 per cent cream.}$$

*Skim milk.* From this ingredient will come the balance of the constituents (m. s. n. f.) of the mix. The amount required will be the difference between the amount of ingredients already used and the total (1750) pounds required. For instance,  $1750 - (245 + 150 + 492.5) = 862.5$  pounds of skim milk.



## EXAMPLE 2

*Mix.* Give the proportions for 500 gallons of ice cream testing approximately 14.5 per cent fat, 13 per cent sugar, 9 per cent milk solids not fat and 0.5 per cent gelatin. The weight of the ice cream desired is 5 pounds per gallon.

*Stock on hand.* Sugar, gelatin, 342 pounds of 30.5 per cent cream, 1608 pounds of 38 per cent cream, 720 pounds of condensed milk testing 8.2 per cent fat, 42 per cent sugar and 21 per cent milk solids not fat, and skim-milk powder.

*Illustration*

TOTAL POUNDS 2500	INGREDIENTS AND COMPO- SITION	FAT: 14.5% 362.5 LBS.	SUGAR: 13% 325 LBS.	M. S. N. F.: 9% 225 LBS.	GELATIN: 0.5% 12.5 LBS.
pounds		pounds	pounds	pounds	pounds
23.0	Granulated sugar		23.0		
12.5	Gelatin (powdered)				12.5
342.0	Cream, 30.5 per cent	104.3		22.0	
524.0	Cream, 38 per cent	199.0		30.0	
720.0	Condensed milk 8.2 per cent fat, 42.0 per cent sugar, 21.0 per cent m. s. n. f.	59.0	302.0	151.0	
23.0	Skim-milk powder			22.0	
855.5	Water				
2500.0		362.3	325.0	225.0	12.5

*Particulars concerning example 2*

The calculations necessary in determining the proportions are as follows (consider the ingredients as they are listed):

*Granulated sugar.* The amount of granulated sugar can not be determined until the condensed milk is proportioned.

*Gelatin (powder).* The amount of gelatin is the same as that calculated for the mix.

*Cream (30.5 per cent).* The 340 pounds of 30.5 per cent cream does not contain more than a small proportion of the fat required, hence the entire amount can be used.

*Cream (38 per cent).* The amount of this cream required can not be proportioned until after the condensed milk is proportioned since it contains 8.2 per cent fat.

*Condensed milk.* The amount of sweetened condensed milk that can be used is limited by the amount of sugar and milk solids not fat it adds to the mix. The 720 pounds of condensed milk will add only 302 pounds of sugar and 151 pounds of milk solids not fat, hence the entire amount can be used.

*Granulated sugar.* With the condensed milk proportioned, the amount of granulated sugar necessary can be determined by subtracting the amount added in the condensed milk from the total amount required, thus:

$$325 - 302 = 23 \text{ pounds of granulated sugar.}$$

*Cream (38 per cent).* Now that the condensed milk is proportioned, the amount of 38 per cent cream may also be determined. The amount is obtained by subtracting the sum of the fat contained in the 342 pounds of 30.5 per cent cream and the 720 pounds of 8.2 per cent condensed milk from the total amount required and divide the remainder by 0.38, thus:

$$\begin{aligned} 362.5 - (104.3 + 59.0) &= 199 \\ 199.0 \div 0.38 &= 524 \text{ pounds of 38 per cent cream.} \end{aligned}$$

*Skim milk powder.* From this ingredient must come the balance of the milk solids not fat needed in the mix. This is determined by the difference between the sum of the m. s. n. f. added by the cream<sup>1</sup> and condensed milk and the total amount required plus 5 per cent.<sup>2</sup> For instance:

$$\begin{aligned} 225 - (22 + 30 + 151) &= 22. \\ 22 + (0.05 \times 22) &= 23.1 \end{aligned}$$

<sup>1</sup> The amount of m. s. n. f. in the cream is determined by multiplying the difference between the amount of cream used and the amount of fat it contains by 0.093 (the amount of m. s. n. f. in the milk serum).

<sup>2</sup> Skim milk powder contains on an average 3.5 per cent moisture and 1.5 per cent fat, consequently an allowance of 5 per cent is made in balancing the m. s. n. f.

*Water.* The required amount of solid constituents having been added, the amount of water needed will be the difference between the total amount of mix required and the sum of the ingredients used.

The accuracy of the calculations can be ascertained by comparing the sum of the figures in each column with the stipulated amounts placed at the top of each column.

When this is done, the ingredients are proportioned by careful weighing. The mix is then ready to be pasteurized and homogenized.

### EXAMPLE 3

*Mix.* Give the proportions for 350 gallons of frozen product testing approximately 9 per cent fat, 14 per cent sugar, 12 per cent milk solids not fat, and 0.5 per cent gelatin. The weight of the product desired is 5 pounds per gallon.

*Stock on hand.* Sugar, gelatin, 150 pounds of 28 per cent cream, 480 pounds of 34 per cent cream, skim milk, and 900 pounds of condensed skim milk.

#### Illustration

TOTAL POUNDS 1750	INGREDIENTS AND COMPOSITION	FAT: 9% 157.6 LBS.	SUGAR: 1.4% 245 LBS.	M. S. N. F.: 12% 210 LBS.	GELATIN: 0.5% 8.75 LBS
pounds		pounds	pounds	pounds	pounds
245.0	Cane sugar		245		
87.5	Gelatin solution, 10 per cent				8.75
150.0	Cream, 28 per cent	42.0		10.0	
340.0	Cream, 34 per cent	115.6		20.8	
397.0	Skim milk, 9 per cent			35.8	
530.0	Condensed skim milk, 27 per cent			143.0	
1749.5		157.6	245	209.6	8.75

#### Particulars concerning example 3

The calculations necessary in determining the proportions are as follows (consider the ingredients as they are listed):

*Sugar.* The amount of sugar is the same as the amount calculated for the mix since there is no cane sugar in the other ingredients.

*Gelatin.* The amount of gelatin solution is determined by moving the decimal point one place to the left, since the solution is a 10 per cent mixture.

*Cream (28 per cent).* The 150 pounds of 28 per cent cream does not contain more fat than is needed, hence the entire amount can be used.

*Cream (34 per cent).* The amount of 34 per cent cream can be determined by subtracting the amount of fat added by the 150 pounds of 28 per cent cream from the total amount required and dividing the remainder by 0.34, thus:

$$157.5 - 42 = 115.5$$

$$115.5 \div 0.34 \times 100 = 340 \text{ pounds of 34 per cent cream.}$$

*Skim milk and condensed skim milk.* From these two ingredients must come the balance of the constituents (m. s. n. f.) of the mix. To find the proportions subtract the sum of the m. s. n. f. in the cream from the total amount required and divide by 927.5 the difference between the amount of ingredients already used and the total (1750) pounds required.

For instance,

$$210 - (10 + 20.8) = 179.2$$

$$179.2 \div 927.5 \times 100 = 19.3 \text{ per cent solids.}$$

This gives the per cent of solids not fat that the additional 927.5 pounds of mix must contain. To find the proportion of skim milk and condensed skim milk necessary, the "square method" is used.

The calculations for the square method are as follows:

Skim milk 9..... 7 7

.	.
.	.
.	.
.	.
.	.
.	.
.	.

19.3

27 ..... 10.3

18.0 total number of parts

$$927.5 + 18 = 51.53$$

$$51.53 \times 7.7 = 396.78 \text{ pounds of skim milk}$$

$$51.53 \times 10.3 = 530.75 \text{ pounds of condensed skim milk}$$

The accuracy of the calculations can be ascertained by comparing the sum of the figures in each column with the stipulated amounts placed at the top of each column.

When this is done the ingredients are proportioned by careful weighing. The mix is then ready to be pasteurized and homogenized.

#### EXAMPLE 4

*Mix.* Give the proportions of the following ingredients necessary for 280 gallons of a frozen product testing approximately 10 per cent fat, 8 per cent sugar, and the equivalent of 6 per cent additional sugar in the form of maltose sugar syrup and corn syrup,<sup>3</sup> 10 per cent milk solids not fat, and 0.5 per cent gelatin. The weight of the product desired is 4.5 pounds per gallon.

*Stock on hand.* Sugar, gelatin, maltose sugar syrup and corn syrup, cream (221 pounds of 40 per cent, 80 pounds of 35 per

#### Illustration

TOTAL POUNDS DESIRED, 1260	INGREDIENTS AND COMPO- SITION	FAT: 10% 126 LBS.	SUGAR: 8% 101 LBS.	M S N. F.: 10% 126 LBS.	GELATIN: 0.5% 6.3 LBS.
pounds		pounds	pounds	pounds	pounds
63.0	Granulated sugar (cane)				6.3
	Gelatin solution, 10 per cent				
151.0	Syrup, 80 per cent solids				
80.0	Cream, 35 per cent	28.0			
82.0	Cream, 24 per cent	19.6			
78.0	Cream, 29.5 per cent	23.0			
64.0	Cream, 28 per cent	17.9			
73.0	Cream, 19 per cent	13.8			
59.0	Cream, 40 per cent	23.6			
252.5	Condensed skim milk, 25 per cent m.s. and 40 per cent sugar		101.0	63.0	
35.5	Skim milk powder			34.0	
322.0	Water				
1260.0		125.9	101.0	125.8	6.3

<sup>3</sup> Maltose sugar syrup and corn syrup are only half as sweet as cane sugar; consequently to replace the 6 per cent sugar it is necessary to use 12 per cent syrup. The solids in the syrup weigh about 121 pounds.

cent, 82 pounds of 24 per cent, 78 pounds of 29.5 per cent, 64 pounds of 28 per cent, and 73 pounds of 19 per cent), condensed sweetened skim milk testing 25 per cent m. s. n. f. and 40 per cent sugar, and skim milk powder.

*Particulars concerning example 4*

The calculations necessary in determining the proportions are as follows (consider the ingredients as they are listed):

*Granulated sugar.* The amount of granulated sugar can not be determined until the condensed milk is proportioned.

*Gelatin.* The amount of gelatin solution is determined, as in example 3, by moving the decimal point one place to the left, since the solution is a 10 per cent mixture.

*Syrups.* The amount of syrup is determined by multiplying 1260 by the percentage desired, thus:

$$1260 \times 0.12 = 151.2 \text{ pounds of syrup.}$$

*Cream.* Since all the different lots of cream are used except the lot testing 40 per cent, the sum of the first five lots will add 102.3 pounds of fat to the mix and the balance is determined by dividing the difference between 126 pounds and 102.3 pounds by the percentage of fat in the sixth lot, thus:

$$\begin{aligned} 126.0 - (28 + 19.6 + 23 + 17.9 + 13.8) &= 23.6 \\ 23.6 \div 0.40 &= 59.0 \text{ pounds of 40 per cent cream.} \end{aligned}$$

*Condensed skim milk.* The amount of condensed milk that can be used is limited by the amount of sugar it will add to the mix. Dividing the amount of sugar needed in the mix by the per cent of sugar in the condensed milk will give the amount of condensed milk that can be used, thus:

$$101 \div 0.40 = 252.5 \text{ pounds of condensed skim milk.}$$

*Granulated sugar.* Since the required amount of sugar is added with the condensed milk, no granulated sugar is needed.

*Skim milk powder.* The amount of skim milk powder is determined by subtracting the sum of the m. s. n. f. added by

the cream<sup>4</sup> and the condensed milk from the total amount required plus 5 per cent<sup>5</sup> thus:

$$126 - (28.8 + 63) = 34$$

$$34 + (34 \times 0.05) = 35.7$$

*Water.* The required amount of solid constituents having been added the amount of water needed will be the difference between the total amount of the mix required and the sum of the ingredients used.

The accuracy of the calculations can be ascertained by comparing the sum of the figures in each column with the stipulated amounts placed at the top of each column.

When this has been done the ingredients are proportioned by careful weighing. The mix is then ready to be pasteurized and homogenized.

#### EXAMPLE 5

*Mix.* Give the proportions for 220 gallons of a frozen product testing approximately 10 per cent fat, 14 per cent sugar, 10 per cent milk solids not fat, and 0.5 per cent gelatin. The weight of the product desired is 4.5 pounds per gallon.

*Stock on hand.* Sugar, gelatin, cream 33 per cent, condensed milk testing 10 per cent fat, 22 per cent m. s. n. f. and whole milk testing 3.6 per cent fat.

#### Illustration

TOTAL POUNDS, 990	INGREDIENTS AND COMPO- SITION	FAT: 10 % 99 LBS.	SUGAR: 14 0% 138 5 LBS.	M. S. N. F. : 10% 99 LBS.	GELATIN: 0.5% 4.95 LBS.
<i>pounds</i>		<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
138.5	Sugar		138.5		
49.5	Gelatin solution, 10 per cent		.		4.95
184.0	Cream, 33 per cent	60.7	}	44.5	
368.0	Whole milk, 3.6 per cent	13.2			
250.0	Condensed milk, 10 per cent fat, 22 per cent s. n. f.	25.0		55.0	
989.0		98.9	138.5	99.5	4.95

<sup>4</sup> The amount of m. s. n. f. in the cream is determined by multiplying the difference between the amount of cream used and the amount of fat it contains by 0.093.

<sup>5</sup> Skim milk powder contains on an average 3.5 per cent moisture and 1.5 per cent fat, consequently an allowance of 5 per cent is made in balancing the m. s. n. f.

*Particulars concerning example 5*

The calculations necessary in determining the proportions are as follows (consider the ingredients as they are listed):

*Sugar.* The amount of sugar is the same as the amount calculated for the mix, since there is no cane sugar in the other ingredients.

*Gelatin.* The amount of gelatin solution is determined by moving the decimal point one place to the left, since the solution is a 10 per cent mixture.

*Cream (33 per cent).* The amount of cream can not be proportioned until after the condensed milk is proportioned, since it contains 10 per cent fat.

*Whole milk (3.6 per cent.)* Temporarily omitted for the same reason.

*Condensed milk.* The amount of condensed milk necessary in this case is determined by using a rough estimate (see page 451). From this estimate it is found that 250 pounds is about the correct amount, thus:

$$990 - (138.5 + 49.5 + 250) = 552$$

$$552 - (99 - 25.0) = 478$$

$$478 \times 0.093 = 44.5$$

$$44.5 + 55 = 99.5 \text{ pounds of milk solids not fat.}$$

*Cream (33 per cent) and whole milk (3.6 per cent).* From these two ingredients must come the balance of the constituents (fat and solids not fat) of the mix. To find the amount of each, subtract the amount of fat added by the condensed milk from the total amount required and divide by 552, the difference between the amount of ingredients already used, and the total (990) pounds required, thus:

$$99 - 25 = 74$$

$$74 \div 552 \times 100 = 13.4 \text{ per cent fat in 552 pounds of milk.}$$



This gives the per cent of fat that the additional 552 pounds of mix must contain.

To find the proportions of cream and whole milk that are necessary the "square method" is used.

The calculations for the "square method" are as follows:

33	.....	9.8
.		.
.		.
.	13.4	.
.		.
.		.
3.6	.....	19.6
		<hr style="width: 100px; border: 0.5px solid black;"/>
		29.4 total number of parts

552	÷ 29.4 =	18.78	
18.78 × 9.8 =		184.0	pounds of cream
18.74 × 19.6 =		368	pounds of whole milk

The accuracy of the calculations can be ascertained by comparing the sum of the figures in each column with the stipulated amounts placed at the top of each column.

When this has been done the ingredients are proportioned by careful weighing. The mix is then ready to be pasteurized and homogenized.

#### ROUGH ESTIMATES

Whenever a mix is made from an unlimited quantity of condensed whole milk the amount of condensed milk required is determined by first making a rough estimate. For instance, in example 5, we do not know what part of the total amount of milk solids not fat of the mix must come from the condensed milk, so we try what we think is about the right amount. In this case the figure taken to begin with was 220 pounds. This figure is taken because from experience we know that about 50 per cent of the m. s. n. f. in the mix must come from the condensed milk. That quantity divided by 22 (the per cent of m. s. n. f. in the condensed milk) shows that it will require about 220 pounds of the condensed milk. This amount would add 24.2 pounds of fat and 48.4 pounds of m. s. n. f. to the mix.

To tell whether or not this is right simply take the difference between the total amount of ingredients already calculated (that is, the pounds of sugar, gelatin, and condensed milk) and the total weight of the mix and subtract the difference between the fat used in the condensed milk and the total amount required to find the amount of milk serum. Then multiply this figure by 9 to get approximately the amount of milk solids not fat that will come from the milk and cream, and the sum of the two will indicate whether the proportions are correct, thus:

$$990 - (138.5 + 49.5 + 220) = 542 \text{ pounds of milk and cream}$$

$$542 - (99 - 24.2) = 466.0 \text{ pounds of milk serum}$$

$$466 \times 0.093 = 43.3 \text{ pounds of m. s. n. f. from serum}$$

$$43.3 + 48.4 = 91.7 \text{ pounds of m. s. n. f. in mix.}$$

The total amount of m. s. n. f. lacks about 7 pounds, so we increase the amount of condensed milk 30 pounds, or to 250 pounds, which gives practically the right amount, as shown in the table.

In case the quantity had been increased only by 20 pounds, the figures would show about 3 pounds of m. s. n. f. less than was desired. The amount of milk and cream is then calculated as heretofore explained.

# A STUDY OF THE INCORPORATION OF PROTEINS IN CREAMERY BUTTER

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## REASONS FOR MAKING STUDY

For many years creamery buttermakers have been operating upon a single standard for determining the composition of butter. This standard, as established by the Internal Revenue Department, fixed the maximum moisture content of butter below 16 per cent (1). The moisture content was taken as a standard for two reasons: first, because moisture is the least valuable of the butter constituents, and second, the composition of butter, so far as moisture is concerned, is largely under control of the buttermaker. Therefore, a standard which limited the amount of moisture in butter was deemed adequate for the protection of the consumer.

The many supporters of a fat standard regulation maintain, however, that the consumer of butter is paying for the fat which it contains and, therefore, the per cent of fat in butter is of greater importance to the consumer than the per cent of moisture. The logic of the foregoing statement is apparent and without doubt the moisture standard in addition to the fat standard will be finally adopted in all countries where the dairy industry is prominent.

The cost of butterfat has doubled in the past three years. Added to the increased cost of raw material is the constantly increasing overhead expense of operating a creamery. Under these conditions it is obvious that the creamery manager must use all of the legitimate means at his disposal to make the greatest profit from the butterfat which is delivered at the factory. Moreover, competitive conditions compel the operator of a creamery to manufacture butter the composition of which is as near the legal limits as prudence will allow.

The present known methods for determining some of the constituents of butter are inadequate for general creamery practice. The moisture and salt content may be accurately determined in any creamery by the buttermaker. These tests will in the future, as they have in the past, be the ones commonly used in the creamery laboratory. For technical reasons the direct determination of the fat and the protein content of butter must be left to the chemical laboratory.

A recent ruling by Dr. C. L. Alsberg, Chief of the Bureau of Chemistry, has made the double standard effective. This ruling requires that the maximum moisture content of butter be less than 16 per cent and the minimum fat content be at least 80 per cent. The single and the double standards are apparently substantially equivalent and it would seem that so long as adulterants were not used in the manufacturing process, the single or moisture standard would protect the consumer from an inferior or less valuable product.

As evidence of the fact that the single standard and the double standard are not equivalent in so far as the composition of the butter is concerned, the following paragraph is submitted.

In a letter to the members, G. L. McKay, secretary of the American Association of Creamery Butter Manufacturers, reports a conversation with Dr. Alsberg, Chief of the Bureau of Chemistry, in which he states:

I personally called his attention to the fact that there is at least 25 per cent of the butter now in storage that would be lower than 80 per cent fat, and it would be a serious problem for the dairy business to have his department go out and seize this butter.

Without question this storage butter referred to by Mr. McKay was legal butter as defined under the moisture standard regulation, that is, this butter contained less than 16 per cent moisture. Moreover, this butter represented normal creamery butter manufactured according to modern methods. These facts suggest that the percentage of the constituents of butter other than fat and moisture is of considerable importance.

Butter contains the following commercial constituents: fat moisture, salt, and curd. The fat content and the moisture content are fixed at 80 and 16 per cent respectively. The other constituents of butter must come within the remaining 4 per cent provided the law is not violated or good factory practice abused. A moderately salted butter contains about 3 per cent salt. Some markets prefer a heavier salt while others demand a light salt or none at all. The average salt content of 695 samples of American creamery butter as reported by the Bureau of Animal Industry (2) was 2.15 per cent. This would still leave 1.85 per cent for the curd content. Keeping the other factors constant, 3 per cent salt would leave 1 per cent for curd. This is in agreement with the analyses of the 695 samples mentioned above as the average curd content of this butter was 0.88 per cent. The fact that of the 695 samples of creamery butter analyzed the curd content of individual samples varied from 0.12 per cent to 3.41 per cent makes this problem worthy of study.

All authorities agree that a high curd content should be avoided so as to insure better keeping quality of the butter. For these two reasons this study was undertaken. It was hoped that the extent of incorporation and the factors that are responsible for incorporation might be determined under factory conditions.

#### REVIEW OF LITERATURE

There is an abundance of published data relating to the composition of creamery butter. The most comprehensive study was completed in 1912 by Thompson, Shaw, and Norton (2) of the Dairy Division and published by the Bureau of Animal Industry. A summary of this bulletin is given on the preceding page. Lee and Barnhart (3) of the Illinois Experiment Station made an extended study of the composition of creamery butter collected from the markets at Chicago, Elgin, and Aurora. These investigators found the average casein and ash content of the 231 samples analyzed to be 0.37 per cent.

Nothing would be gained in this study by reviewing the entire published data upon the composition of creamery butter. Most

of the analyses were made to determine the moisture content as the moisture standard was effective at that time. The curd content of butter has been for the most part ignored. It has been variously reported by investigators as casein, protein, nitrogen, and curd. The per cent of this constituent in butter was often arrived at by subtracting the sum of the percentage of fat, moisture, and salt from one hundred.

Attention might be called to the results of the analyses of 200 samples of Minnesota creamery butter as reported by James Sorensen, State Dairy and Food Commissioner (4), and tabulated by McKay (5). The curd content of this butter, calculated by difference, varied from nothing to 10.42 per cent.

The following review of literature is not by any means exhaustive. The results of the principal investigations upon the relation of curd to the keeping quality and composition of butter are given together with comments by several of the leading authorities.

Guthrie (6) states, "The amount of washing that butter receives and the quality of the wash water is important. From the physical standpoint butter must be washed because the brine should be clear and not milky. From the bacteriological viewpoint the buttermilk should be washed out so that the bacteria will be deprived of it as a food."

According to Jensen (7) "lactic acid bacteria were found to multiply much more rapidly in unwashed than in washed butter."

Thom and Shaw (8) add that "excess curd in butter favors mold growth and if the butter is properly washed it is less subject to the mold."

McKay and Larsen (9) after extended study and observation conclude that "butter should not be over churned in the buttermilk as too much curd and milk sugar are incorporated. This cannot readily be removed and in many instances it injures the flavor and keeping quality of the butter."

McKay and Larsen further discuss the effect of curd upon the keeping quality and composition of butter:

According to the present methods of manufacture, water, salt, and fat are the components most likely to vary. Casein varies very little. Occasionally the curd content may go as high as 4 per cent. It rarely exceeds 2 per cent and seldom falls below 0.5 of 1 per cent. A high curd content will show itself in the butter in the form of a milky brine or in the form of white specks. If there is less than 2 per cent curd present in the butter, the brine shows no noticeable milkiness. More than that much curd can, as a rule, be detected from the color of the brine. If the casein or the curd has been incorporated in the form of small lumps or specks, then abnormal amounts of curd appear. When the sample of butter is taken for analysis such a speck of curd present in the sample raises the final curd content to a comparatively high figure. As has been mentioned before, the curd and milk sugar are incorporated from the buttermilk into the butter during the churning. In manufacturing butter for storage these substances should be excluded from the butter as thoroughly as possible. The milk sugar and albuminoids constitute the chief food for bacterial growth.

Michels (10) believes "one washing in which as much water is used as there was cream is usually sufficient. When butter churns very soft two washings may be advantageous."

Wing (11) advances a reason for the variation in casein content by stating the "higher the temperature at which the cream is churned, the more casein will be incorporated with the butter." Wing concludes without giving any reason that the percentage of casein in butter should not exceed 4 per cent.

Mortensen, Gaessler, and Cooper (12) believe "the pasteurization of sour cream affects the percentage of protein in the butter, as the casein in the presence of the acid is hardened and thrown into clumps known as curd particles that are quite readily removed in the draining and washing of butter."

Stocking (13) concludes "the purpose of washing butter is to remove the buttermilk" without giving any reason either for or against a small amount of curd in the finished butter.

Hunziker (14) concludes:

In the case of the true curd of butter the percentage of curd fluctuates between about 0.5 per cent and one per cent, averaging about from 0.6 to 0.7 per cent, provided that the butter is washed in a normal

manner. Butter made from sweet cream and unsalted butter, has a slightly higher curd content than butter made from ripened cream and butter that was salted. Butter that is only very slightly washed contains more curd than butter in the manufacture of which the churn is stopped when the granules are very small and which is washed very thoroughly. Butter that is not washed at all usually contains from about 1 to 1.5 per cent curd.

#### THEORETICAL DISCUSSION

The principal nitrogenous compounds of milk are casein and albumin. There are, however, several other proteins in milk studied by Storch (15) which have properties unlike either casein or albumin. These occur in very small amounts and are, therefore, not of great importance commercially. The albumin of milk is in a true solution (16). It likewise occurs in small amounts and is not of commercial importance. The chief nitrogenous compound of milk is the casein. It exists in milk in combination with certain calcium salts in the form of extremely minute gelatinous particles in suspension. Casein is insoluble in water and is precipitated by heat in the presence of dilute acids or acid salts (17). In the process of cream separation most of the casein is thrown out as skim milk. The remaining portion is thrown out with the fat and these two constituents with the lactose make up the principal solid material in cream. A cream low in fat must, therefore, be high in casein.

The preliminary treatment cream receives in the pasteurization and ripening process before churning tends to precipitate the casein. The degree of precipitation depends upon the duration and intensity of the heat used and the acidity of the cream (14). Thus when the cream is placed in the churn the casein is in a fine granular condition and with the fat globules forms an emulsion (18).

As the cream is agitated during the churning process the individual fat globules coalesce forming a globule of larger size. A continuation of the agitation forms butter granules and when the granules are about 50  $\mu$ m. in diameter the churning is complete. While the granule is forming small particles of casein or other



nitrogenous compounds are mechanically incorporated which cannot be removed by subsequent washing. The casein that adheres to the outside of the granule or is held mechanically in pockets between the granules may be removed by careful washing and draining.

Casein particles may be present in butter in two forms. The small minute particles, referred to in the preceding paragraph, are invisible and can only be seen when large numbers are suspended in water, forming a milky brine. Under certain conditions of manufacture where the cream is allowed to sour and become lumpy and is then pasteurized, the lumps of curd are hardened, due to the action of heat in the presence of acid. These lumps may remain intact during churning and be finally incorporated, producing the characteristic "speckled" butter sometimes found on the market. This butter shows defective workmanship and would hardly be termed normal because modern buttermakers know how to avoid this condition. Without question some of the analyses showing a high percentage of curd were made from this inferior product. In this study only butter made according to present day knowledge will be considered normal.

In a previous paragraph the fact that some confusion exists as to the definition of the word curd is mentioned. Curd is usually defined as the remaining constituents of butter which are not included under the terms fat, moisture, and salt. It consists mainly of casein, albumin, lactose, lactic acid, and ash. McKay and Larsen (9) state "in the analysis of butter the milk sugar is usually included with the proteids (curd) and the ash is reckoned with the salt."

Guthrie (6) says, "The curd of modern butter contains very little if any albumin, for it is taken out in the washing. The curd, therefore, is largely casein."

Lee and Barnhart (3) express this constituent of butter as casein and ash. Mortensen, Gaessler, and Cooper (12) report this constituent as protein. Thom and Shaw (8) state "The curd is equal to the nitrogen times the factor 6.38." Thompson, Shaw, and Norton (2) state, "The term curd includes the lactose and ash."

It will be seen from the foregoing statements that an accepted or recognized definition of curd has not been established. For this reason the term protein (nitrogen times 6.38) is used throughout this paper.

#### EXPERIMENTAL WORK

In order to determine the extent that the nitrogenous compounds of milk may be incorporated in butter under factory conditions a number of churnings were made. By varying the methods of churning, washing, and draining an attempt was made to find the amount of nitrogenous substances which might be added to the butter and the conditions which influence their incorporation.

One vat of cream was used for each experiment. After the preliminary vat treatment, as outlined in the vat record, the cream was divided into two churnings. One churning was handled according to good factory methods and served as a check, being normal creamery butter. The remaining portion of the cream was handled so as to make a possible variation in the protein content of the finished butter. The following variations from the normal were used:

1. Unwashed butter.
2. Working butter in buttermilk.
3. Over churning in the presence of buttermilk.
4. Churning at high and low temperatures.
5. Adding starter after churning.

A vat and churn record was completed for each experiment. The duplicate lots of butter were sent to market to find if any variation in score or selling price of the butter would be made.

#### METHOD OF SAMPLING

When the butter was completely finished and ready to put into tubs small samples were taken from at least twelve parts of the churn. These were placed in a glass jar and covered. The covers were made air tight by dipping in melted paraffin. The samples were then held at 10°F. until analyzed. When

ready for analysis the jars were placed in a water bath at 75–80°F. until the butter was soft enough so that it could be thoroughly stirred and mixed until of salvy consistency.

#### METHOD OF ANALYSIS

A number of preliminary samples were analyzed to determine the protein content according to official and optional methods. The optional method recommended by Leach (19) was finally adopted for this work. This method was as follows: About 10 grams of the properly mixed sample were weighed on an analytical balance into a tared, flat aluminum moisture dish. The moisture was evaporated from the sample by heating on a hot plate at 100°C. until bubbling ceased. The contents of the dish were then cooled and 50 cc. of petroleum ether were added. The contents of the dish were next washed with petroleum ether on to a filter paper and the residue washed approximately free from fat with repeated washings of petroleum ether. The residue was then allowed to dry on the filter paper which was then transferred to a Kjeldahl digestion flask and the nitrogen determined according to the Gunning (20) method. The protein was calculated as follows:

$$\frac{\text{Nitrogen} \times 6.38}{\text{Amount of sample}} \times 100 \text{ equals per cent of protein.}$$

In all cases triplicate determinations of each sample were made.

#### METHOD OF CHURNING

The normal churnings in the following experiments were made according to a standard method and represent butter manufactured under modern factory conditions. As these churnings were made during the winter and spring months a variation in churning temperature was necessary, due to the seasonal change in the composition of butterfat. The chief variation between the normal and special churnings was in the washing, working, and draining of the butter. The standard method used in all normal churnings was as follows: When the butter granules were

about 50 mm. in diameter the buttermilk was drawn off and, using a hose, water was sprayed over the butter and allowed to drain out through the open churn gate. Enough wash water was used in this manner to free the churn and butter granules from excessive buttermilk. When the wash water was but slightly turbid the churn gate was closed and enough water was added to the churn to float the butter. The churn was revolved several times, without the workers, to wash the butter granules. The workers were then engaged and the butter worked in the wash water several revolutions of the churn. The water was drawn off first through the churn gate and finally carefully drained through the partly opened churn cover. The butter was then tested for moisture, salted, and finished.

The special churnings in this experiment (table 1) were handled in the same manner as the normal churnings until the churning process was complete. The buttermilk was then drawn off through the churn gate as completely as possible and the butter carefully drained from unincorporated buttermilk by revolving the churn several times and allowing the buttermilk to drain out of the partly opened churn cover after each revolution. The butter was then salted and finished without washing. The butter of the special churnings compared favorably as to flavor, body and color with the butter of the normal churnings which were made from the same vat of cream.

The special churnings in this experiment (table 2) were handled to the end of the churning process in the same manner as the normal churnings. Then one-half of the buttermilk was drawn off and the butter worked in the buttermilk. The remaining buttermilk was drawn off as thoroughly as possible through the churn gate and then carefully drained by revolving the churn several revolutions and allowing the buttermilk to drain out through the partly opened churn cover. When most of the unincorporated buttermilk had been removed in this manner, the butter was salted and finished without washing. The body and flavor of the butter in the special churnings were similar to that of the normal churnings which were made from the same vat of cream.

TABLE 1  
*To determine the effect of washing upon the protein content of butter; vat and churn record*

	VAT 1		VAT 2		VAT 3		VAT 4	
	Special Good	Normal Good	Special Good	Normal Good	Special Good	Normal Good	Special Good	Normal Good
Churning.....								
Condition of cream.....								
Pounds of cream in churn.....	804	804	797	797	739	739	841	841
Per cent of fat in cream.....	40	40	40	40	34	34	35	35
Per cent of acid before neutralization.....								
Per cent acid at the churn.....	0.42	0.42	0.33	0.33	0.48	0.48	0.47	0.47
Temperature of pasteurization for 30 minutes, °F.....	0.28	0.28	0.33	0.33	0.25	0.25	0.34	0.34
Temperature held in vat, °F.....								
Time held in vat, hours.....	145	145	145	145	145	145	145	145
Churning temperature, °F.....	45	45	44	44	45	45	45	45
Churning time, minutes.....	3	3	2	2	20	20	3	3
Temperature of buttermilk, °F.....	50	50	48	48	53	53	49	49
Times washed.....	20	28	30	30	25	18	45	50
Temperature of wash water, °F.....	57	58	56	56	56	56	55	56
Revolutions worked in wash water.....	None	2	None	2	None	2	None	2
Total revolutions worked.....	58	58	56	56	54	54	56	56
Method of draining.....	None	12	None	7	None	9	None	13
	35	47	15	25	25	35	26	44
Gate and cover	Gate and cover	Gate and cover	Gate and cover	Gate and cover	Gate and cover	Gate and cover	Gate and cover	Gate and cover
Per cent of protein.....	0.5406	0.4012	0.4542	0.3155	0.5505	0.3782	0.5252	0.4372

TABLE 2

*To determine the effect upon the protein content of the finished butter by working the butter in the buttermilk; vat and churn record*

	VAT 1		VAT 2		VAT 3	
	Special Good	Normal Good	Special Good	Normal Good	Special Good	Normal Good
Churning.....	784	784	834	834	694	694
Condition of cream.....	30	30	37	37	42	42
Pounds of cream in churn.....	0 36	0 36	0 36	0 36	0 32	0 32
Per cent of fat in cream.....	0 35	0 35	0 31	0 31	0 33	0 33
Per cent of acid before neutralization .....	145	145	145	145	145	145
Per cent acid at the churn.....	45	45	45	45	45	45
Temperature of pasteurizing for 30 minutes, °F.....	2	2	17	17	20	20
Temperature held in vat, °F.....	49	49	56	56	56	56
Time held in vat, hours.....	25	25	25	25	25	25
Churning temperature, °F.....	58	57	58	58	59	58
Churning time, minutes.....	2	2	None	2	None	2
Temperature of buttermilk, °F.....	56	56	None	58	58	58
Times washed .....	5	5	None	5	None	6
Temperature of wash water, °F.....	10	None	10	None	20	None
Revolutions worked in wash water.....	24	24	40	20	35	19
Revolutions worked in buttermilk .....	Gate and cover	Gate and cover	Gate and cover	Gate and cover	Gate and cover	Gate and cover
Total revolutions worked.....	0 5562	0 4189	0 5064	0 3476	0 5166	0 3676
Method of draining.....						
Per cent of protein.....						

TABLE 3

*To determine the effect of adding starter to the butter upon the protein content of the finished butter; vat and churn record*

	VAT 1	
	Special	Normal
Churning.....	Good	Good
Condition of cream.....	587	587
Pounds of cream in churn.....	38	38
Per cent of fat in cream.....	0.40	0.40
Per cent of acid before neutralization.....	0.30	0.30
Per cent acid at the churn.....	145	145
Temperature of pasteurization for 30 minutes, °F....	45	45
Temperature held in vat, °F.....	16	16
Time held in vat, hours.....	54	54
Churning temperature, °F.....	15	20
Churning time, minutes.....	56	56
Temperature of buttermilk, °F.....	1	2
Times washed.....	56	56
Temperature of wash water, °F.....	None	6
Revolutions worked in wash water.....	10	None
Revolutions worked after adding starter.....	23	36
Total revolutions worked.....	Gate and cover	Gate and cover
Method of draining.....	0.4718	0.3155
Per cent of protein.....		

The special churning in this experiment (table 3) was handled in the same manner as the normal churning until the butter granules were of the desired size. After carefully washing the butter granules with a spray of water from a hose, four gallons of ordinary starter were added to the churn. The butter was then worked ten revolutions of the churn. The excess starter was then drained off and the butter salted and finished without washing. It is apparent that all of the starter which was added could not be incorporated without over working the butter.

In order to determine the effect of abnormally high churning temperature upon the protein content of the finished butter two churnings were made in a ten gallon barrel churn.

In churning number one the cream was pasteurized, ripened, and then churned at 60° F. This butter was carefully worked and drained but was not washed. The body of this butter was

TABLE 4

CHURN NUMBER	PER CENT BUTTER FAT	ACIDITY	CHURNING TIME	MOISTURE	PER CENT PROTEIN
1	34	0.35	25	17	0.4900
2	18	0.80	20	20	0.5915

somewhat weak but represented a good quality of farm dairy butter.

The cream for the second churning was placed at 70° F. for four days and allowed to ripen spontaneously. The cream was pasteurized and then churned at 65° F. When the churning was complete the buttermilk was nearly all removed and the butter was worked to incorporate as much buttermilk as possible. This butter was not washed. The butter in this churning represented an unsatisfactory grade of farm dairy butter.

The number of churnings in the foregoing experiments are not extensive enough to be conclusive but the results obtained from the analysis of the butter were so uniformly constant that further churnings were not deemed necessary.

#### SUMMARY

The average protein content of the normal samples of butter in these experiments was 0.3727 per cent.

The average protein content of the butter which was worked in the buttermilk was 0.5264 per cent.

The average protein content of the unwashed butter was 0.5177 per cent.

The average loss of protein due to washing the butter was 0.1450 per cent.

The protein content of the unwashed butter, churned at abnormally high temperature, was 0.5407 per cent.

The protein content of the butter to which starter had been added was 0.4718 per cent.

Nitrogenous compounds were not incorporated in the butter to any appreciable extent by using the methods of manufacture followed in these experiments.



One per cent was found to be sufficient allowance for all the constituents of butter which may be classified under the term curd.

By incorporating 15.5 per cent moisture and allowing 1 per cent for curd as much as 3.5 per cent of salt may be incorporated without reducing the fat content of the butter below 80 per cent.

No difference in score or selling price of the duplicate lots of butter was made upon the Chicago market.

Speckled butter was not found in any of the samples.

Milky brine was not found in any samples of the finished butter.

With the exception of one churning the keeping quality of the washed butter was slightly better than that of the unwashed butter.

#### CONCLUSION

Excessive overrun is probably due to the incorporation of moisture and not to curd under modern factory conditions.

#### REFERENCES

- (1) United States Department of Agriculture Yearbook. 1913, p. 487.
- (2) THOMPSON, S. C., SHAW, R. H. AND NORTON, R. P. 1912 The normal composition of creamery butter. Bureau of Animal Industry Bulletin 149, p. 3.
- (3) LEE, CARL E., AND BARNHART, JESSE M. 1909 Composition of market butter. Ill. Expt. Station Bulletin 139, p. 450.
- (4) SORENSEN, JAMES 1918 Minnesota butter analyzed. Chicago Dairy Produce, August 6, 1918, p. 32.
- (5) MCKAY, G. L. 1918 Statements on the subject of definitions and standards of butter presented to the joint committee on definitions and standards, p. 90.
- (6) GUTHRIE, E. S. 1916 The Book of Butter, pp. 174, 11.
- (7) JENSEN, O. A. 1910 Bacteriological studies of Danish butter. Rev. Gen. Lait. 8 (1910), No. 18, p. 409.
- (8) THOM, CHAS., AND SHAW, R. H. 1915 Moldiness in butter. Jour. Agr. Res., 3: no. 4, p. 304.
- (9) MCKAY, G. L., AND LARSEN, C. 1910 Principles and Practice of Butter-making, pp. 246, 247, 281, 282.

- (10) MICHELS, JOHN 1904 Creamery Butter-Making, p. 103.
- (11) WING, HENRY H. 1911 Milk and Its Products, pp. 143-147.
- (12) MORTENSEN, M., GAESSLER, W. G., AND COOPER, W. H. 1914 Pasteurization of cream for buttermaking. Iowa Expt. Station Bulletin 156, p. 15.
- (13) STOCKING, WM. A., JR. 1917 Manual of Milk Products, p. 254.
- (14) HUNZIKER, O. F. 1920 The Butter Industry, pp. 219, 547.
- (15) STORCH. Bericht des Koenigl. Veterinar- und Landbanhochschule-Laboratoriums, Kopenhagen, Denmark.
- (16) VAN SLYKE, L. L. 1912 Modern Methods of Testing Milk and Milk Products, p. 11.
- (17) SOMMERS, H. H., AND HART, E. B. 1919 The heat coagulation of milk. Jour. Biol. Chem., 40, no. 1, November, 1919, p. 137.
- (18) FISHER, M. F., AND HOOKER, M. O. 1917 Fats and Fatty Degeneration, p. 19.
- (19) LEACH, A. E. 1914 Food Inspection and Analysis, p. 534.
- (20) GUNNING 1914 Food Inspection and Analysis. Leach, p. 145.

## FACTORS INFLUENCING THE VISCOSITY OF SWEETENED CONDENSED MILK

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In preparing sweetened condensed milk for the market it is essential that the viscosity shall not vary beyond certain limits. It must be sufficiently viscous to give the milk a smooth creamy consistency, but should not become, at any stage, so thick that it will not flow freely. There is, however, a tendency for condensed milk to gradually become thicker on standing until in some cases it acquires a jelly-like consistency and will not flow from the can. Two types of thickening may be distinguished by superficial examination. In one the thickening is not uniform throughout the can but usually begins at the top and gradually extends downward until the entire can is affected. The curd is of a pasty texture and when mixed with water, remains in the form of insoluble flecks. This type of curd is usually associated with unpleasant changes in flavor and odor and is undoubtedly due to the action of bacteria.

In the type of thickening with which this paper is concerned the increase in viscosity proceeds uniformly throughout the can, and there is no marked change in flavor. No real curd is formed and the thickened milk can be mixed with water without the separation of definite flecks. In the earlier stages agitation will restore the milk, partially at least, to its original fluid condition, but in time it takes on a jelly-like consistency which can not be removed. There are many reasons for believing that this slow change which takes place sooner or later in all condensed milk is not due to the growth of bacteria. The fact that no correlated bacterial multiplication has been detected is not conclusive, although it can be admitted as negative evidence. Other evidence which will be brought forward in this paper leads us to

believe that it is a physical change in the milk controlled by various factors in which bacteria have no direct part.

The full explanation of these changes requires a knowledge of the physical chemistry of a highly complex fluid, a knowledge which is still inadequate. It is possible, however, to determine by empirical methods some of the factors influencing these changes without attempting to explain the nature of the change itself.

#### EXPERIMENTAL METHODS

The condensed milk used in these experiments was made for the most part in balloon flasks of about 10 liters capacity. Three of these flasks were suspended in a common water bath and each connected through a condenser and a graduated receiver with a pump giving a vacuum of about 28 inches. Stopcocks permitted the milk to be drawn slowly into the flasks. On the whole the equipment simulated very closely the commercial process, but permitted the manufacture of three batches at once from the same lot of milk with a very accurate control of the product. Knowing the composition of the raw milk, the amount of water to be removed to obtain any desired composition could be calculated and the water distilled measured roughly on the graduated receiver. The final point at which the condensation was stopped was determined by disconnecting the flask from the apparatus and weighing on a balance accurate to 10 grams. The usual batch was 3500 grams of raw milk. Unless otherwise stated skim milk was used, because by eliminating the fat in the milk the product was made slightly less complicated. The milk was produced on the Dairy Division farm and was of good quality.

The finished milk was cooled by rotating the condensing flask slowly in a tank of water starting with the water at the temperature of the milk and cooling it slowly to about 20°C. (68° F.) In addition to this laboratory equipment the laboratory is provided with a small pan having a capacity of about 15 gallons of finished product. This equipment is identical in its arrangement with that of the ordinary condensery except that in place of the

usual spray condenser a surface condenser provides for the collection and measurement of the distillate. The condensed milk was held in ordinary soldered-cap baby-size cans, and was stored at 30°C. (86° F.) This temperature represents the extreme rather than the normal condition, but was adopted because it increased the rapidity of the change and gave quicker results.

The viscosity of the finished milk was measured in a viscosimeter designed by Dr. Clark of this laboratory. It consisted essentially of a heavy brass cylinder suspended by a steel wire in a larger cylinder which was revolved by an electric motor held automatically at a constant speed. The revolving cylinder was water-jacketed and held at a temperature of 40°C. (104° F.) by a thermostat and electric heater. The milk was warmed to 40°C. (104° F.) before it was transferred to the viscosimeter. The rotation of the suspended cylinder due to the viscosity of the milk was read from a pointer moving over a graduated circle, or for the more minute changes by a telescope reading a scale reflected in a small mirror set in the top of the cylinder.

Unless otherwise stated the viscosimeter readings given in the tables are in degrees indicated by the pointer, which have a value about twice the degrees of the telescope scale. The readings have not been standardized and have a relative value only. Normal condensed whole milk should give a reading of 5 to 12 degrees on the pointer or 10 to 24 degrees on the telescope scale.

#### FACTORS WHICH APPARENTLY DO NOT AFFECT VISCOSITY

There are certain steps in the usual process of making condensed milk which might reasonably be expected to have an influence on the viscosity but which so far as we have been able to determine have in fact little or no effect. In consideration of the well-known catalytic action of salts of copper and its detrimental effect in certain other dairy products, it would not be unreasonable to suppose that it would have some influence on condensed milk. Experiments in which the milk was heated by steam forced through a coil of small copper tubing and others in

which copper salts were added directly to the milk failed to show any increase in viscosity over the check.

Sweetened milk is not usually homogenized, but it may be of some interest to state that, while this has the effect of increasing the initial viscosity it is without appreciable effect on the progressive increase in viscosity. This is shown by table 1 which gives the result of an experiment in which one half of a batch of milk was homogenized at 3000 pounds pressure. The initial viscosity of the homogenized lot was higher than the check, but there was no marked difference in the rate at which it increased in the two samples.

TABLE 1

*Effect of homogenizing on viscosity. Milk solids 28 per cent, fat 7.76 per cent, forewarming temperature 63°C. (145.4° F.) storage temperature, 30°C. (86° F.)*

AGE	NOT HOMOGENIZED	HOMOGENIZED AT 3000 POUNDS PRESSURE
days	rotation, degrees	rotation, degrees
1	3.4	8.8
8	3.6	12.8
15	5.0	18.3
31	13.1	18.6

Notwithstanding the fact that the milk is exposed for the greater part of the condensing process to a high vacuum, the milk in the cans contains a considerable proportion of air from which the oxygen disappears in about two weeks. Evidently the oxygen combines with some constituent of the milk and it is not unreasonable to suppose that it may have some effect on the viscosity. We have been unable, however, to find that it has any appreciable effect. Table 2 shows the progressive change in viscosity in a lot of condensed skim milk one-half of which was sealed in cans in the usual way at atmospheric pressure. The remainder was drawn slowly into an evacuated flask to remove the greater part of the contained air, and transferred at once to glass bulbs holding about 150 cc.

These bulbs were evacuated by a Geryk pump which reduced the pressure rapidly to a fraction of a millimeter. At this very low pressure there was considerable ebullition of the milk, due

possibly to the escape of water vapor. The neck of the bulb was sealed off. At 30°C. (86° F.) the change in viscosity was so slight that it could be measured with the telescope throughout the storage period and was practically identical for the bulbs and the cans.

TABLE 2

*Influence of contained air on viscosity. Milk solids 26 per cent, forewarming temperature 63°C. (145.4° F.), storage temperature 30°C. (86° F.)*

AGE	CANS SEALED AT ATMOSPHERIC PRESSURE	EVACUATED BULBS
<i>days</i>	<i>rotation, degrees*</i>	<i>rotation, degrees*</i>
4	2.1	2.1
18	3.1	2.5
33	3.6	2.9
67	4.8	4.3
88	7.5	9.3
116	14.2	13.0

\* Scale.

TABLE 3

*Influence of acidity on viscosity. Milk solids 26 per cent, fat 0, forewarming temperature 63°C. (145.4° F.), storage temperature 30°C. (86° F.). Acidity increased with lactic acid*

BEFORECONDENSING. AFTER CONDENSING..	pH 6.51 6.17	pH 6.41 6.10	pH 6.30 6.00	pH* 6.63 6.36
<i>days</i>	<i>rotation, degrees†</i>	<i>rotation, degrees†</i>	<i>rotation, degrees†</i>	<i>rotation, degrees†</i>
2	3.4	3.0	3.3	3.5
10	3.4	3.1	3.4	4.0
17	3.4	2.8	3.6	4.1
30	3.6	3.6	3.8	4.3
58	6.3	4.1	4.2	

\* Neutralized from 6.30.

† Scale.

The acidity of the milk is another factor which would be expected to influence the viscosity. It has been shown by Wolfgang (1) that very small changes in the hydrogen ion concentration have a marked effect on the viscosity of albumen solutions. Table 3 shows the result of an experiment in which the acidity of the original milk was changed by the addition of

lactic acid. The reaction of the milk both before and after condensing is shown in terms of hydrogen ion concentration as determined for us electrometrically by Dr. Clark of this laboratory. It should be remembered that the acidity varies inversely as the pH of Sorensen's scale. In this experiment the acidity of the milk was increased from a pH of 6.51 as shown in the first column, to 6.30 by the addition of lactic acid. The acidity of the milk shown in the fourth column was neutralized from 6.30 to 6.63 as shown in the last column. The initial viscosity of all of this milk was low and held almost without change for 58 days at 30°C. There was no real difference in the viscosity of the various lots. In one experiment in which the reaction of the milk was very materially changed it became thick in the pan, but we have been unable to find that any variation in the acidity within reasonable limits has an appreciable influence on the viscosity.

The cane-sugar concentration has only a minor influence on the initial viscosity and probably none at all on the progressive increase in viscosity.

This is illustrated by the results shown in table 4 and figure 1. In this experiment the three lots of milk were identical except in the quantity of added sugar. Since the preserving action of the sugar is dependent on the concentration of its aqueous solution and not on its relation to other constituents of the milk, it is given in this table and elsewhere in this paper in terms of its solution. Obviously this is not strictly accurate, because we have no way of knowing what part of the water is available for dissolving the sugar, but on the whole it gives a much more satisfactory indication of the conditions than the usual way of expressing it as a percentage of the milk.

In two weeks the viscosity varied slightly, but in direct proportion to the sugar content. The viscosity of the milk containing 62 per cent and 58 per cent sugar solution increased slightly in thirty-two days but maintained their initial relation. On the other hand the milk containing a 54 per cent sugar solution increased in viscosity slowly at first but with increasing rapidity until at thirty-two days it was a firm jelly. The odor



and flavor of this milk as well as the nature of the curd indicated that the low sugar concentration permitted the growth of bacteria which, no doubt, accounted for the greatly increased viscosity.

TABLE 4

*Influence of cane-sugar concentration on viscosity. Milk solids 28 per cent, fat 0, forewarming temperature 48°C. (118.4° F.), storage temperature 30°C. (86° F.)*

AGE	CANE SUGAR CONCENTRATION		
	54 per cent	58 per cent	62 per cent
days	rotation, degrees	rotation, degrees	rotation, degrees
1	1.75	2.0	4.0
13	2.5	3.0	7.5
21	10.25	4.25	7.5
32	38.0	9.0	11.0

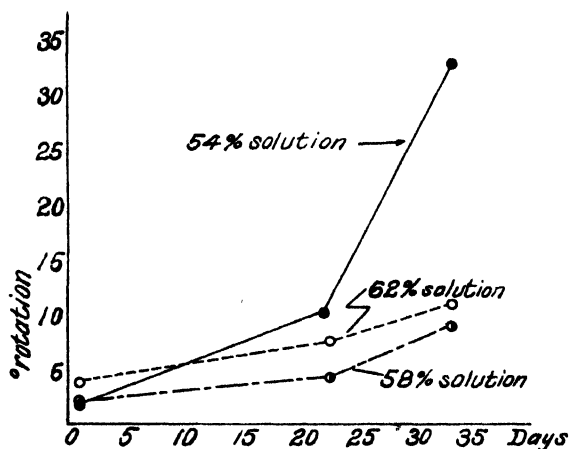


FIG. 1. THE RELATION OF CANE SUGAR CONCENTRATION TO VISCOSITY

#### INFLUENCE OF STORAGE TEMPERATURE ON INCREASE IN VISCOSITY

No special effort is made to store condensed milk at low temperatures, and in its transportation and sale it is frequently exposed to summer heat for extended periods. It is to be expected that this would tend to thicken the milk., In table 5

TABLE :

*Influence of storage temperature on viscosity. Total solids 71.77 per cent, milk solids 28.57 per cent, fat 8.2 per cent, forewarming temperature 75°C. (161° F.)*

AGE	STORAGE TEMPERATURE			
	10°C. (50° F.)	20°C. (68° F.)	30°C. (86° F.)	37°C. (98.6° F.)
days	rotation, degrees	rotation, degrees	rotation, degrees	rotation, degrees
1	2.5	2.5	2.5	2.5
8			3.0	
11	2.5	3.0		12.0
21	2.5	4.0	7.0	18.0
31	2.5	4.5	9.0	20.0
59	2.5	10.0	15.0	37.0
94	2.5	13.0	16.5	38.0
121	2.5	13.5	16.7	39.0

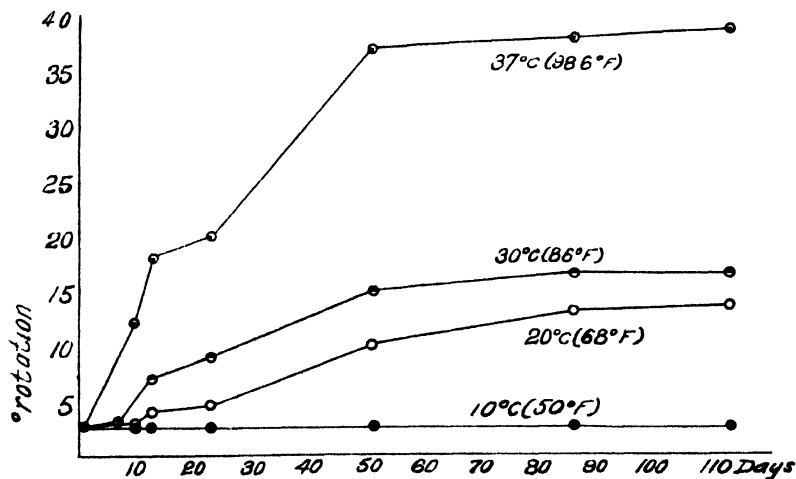


FIG. 2. INFLUENCE OF STORAGE TEMPERATURE ON VISCOSITY

and in fig. 2 are shown the changes in viscosity in whole milk held for 121 days at various temperatures. It is interesting to note that the milk held at 10°C. (50°F.) remained unchanged throughout this period. There was a slow increase in viscosity at 20°C. (68° F.) and at 30°C. (86° F.). An additional 7° made a much greater difference in the viscosity than the 20° change

from 10°C. (50° F.) to 30°C. (86° F.). The nature of the thickening in the milk held at 37°C. (98.6° F.) did not suggest bacterial fermentation, nor have we been able to find by plating any evidence of bacterial growth which would account for this change.

#### THE INFLUENCE OF THE COMPOSITION OF THE MILK ON VISCOSITY

It is a matter of common knowledge that if milk is concentrated beyond a certain point it will become thick in a short time. The relation of the concentration of the milk solids of

TABLE 6

*Relation of concentration of milk solids to viscosity. Skim milk, forewarming temperature 80°C. (176° F.); storage temperature 30°C. (86° F.)*

AGE	MILK SOLIDS NOT FAT		
	24 per cent	26 per cent	28 per cent
days	rotation, degrees	rotation, degrees	rotation, degrees
1	2.0	3	5.25
12	3.0	10	25.0
24	5.0	19	130.0
34	7.5	25	Beyond range

TABLE 7

*Relation of concentration of milk solids to viscosity. Skim milk, forewarming temperature 60°C. (140° F.); storage temperature 30°C. (86° F.)*

AGE	MILK SOLIDS NOT FAT		
	20 per cent	24 per cent	28 per cent
days	rotation, degrees	rotation, degrees	rotation, degrees
1	0.5	1.25	2.25
13	1.0	1.0	2.5
25	1.0	1.0	4.0
35	1.0	1.0	8.0

skim milk to the increase in viscosity is shown in table 6. The tendency to become thick on standing is evidently not in a direct relation to the concentration of the milk solids but increases more rapidly as the concentration becomes greater. This relation varies with different milks, but we have always found a greatly increased tendency to thicken in passing from 26 to 28 per cent milk solids in skim milk.

It became evident very early in this work that factors other than the concentration of the milk solids had an important influence on the viscosity. Table 7 shows the changes in viscosity of three batches of milk treated in every way the same as

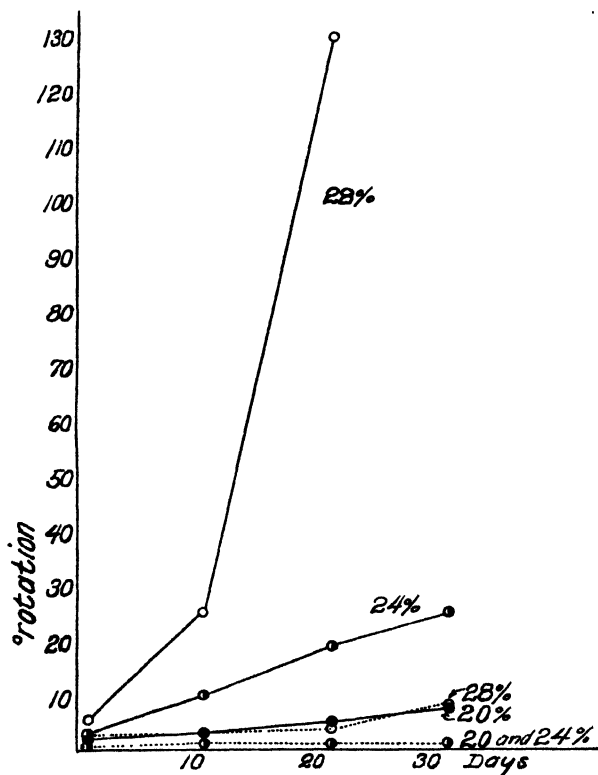


FIG. 3. INFLUENCE OF CONCENTRATION AND FOREWARMING TEMPERATURE ON VISCOSITY

Solid lines: forewarmed to 80°C. (176° F.)

Dotted lines: forewarmed to 60°C. (140° F.)

those in table 6, except that those in table 6 were forewarmed to 80°C. (176° F.), while those in table 7 were not heated above 60°C. (140° F.) The very marked difference in these milks is brought out more clearly in figure 3, in which the viscosity of

the milk with high forewarming is shown by solid lines while the low-forewarmed milk is shown by broken lines. This effect of high forewarming has been shown by repeated experiments, although the increase in viscosity due to high heating has not always been so marked as in the experiment cited in this table.

Not all of the constituents of the milk are concerned in this change in viscosity. The fat exists as very small globules floating in the serum and in all probability is not a factor in determining the viscosity. It is difficult to vary the fat in a normal manner and at the same time hold the other constituents constant. Two experiments in which the fat was varied in different ways indicated that it was not an important factor in the viscosity.

This is also true of the lactose. Any variation of this constituent within reasonable limits has only a minor effect.

TABLE 8

*Relation of casein and albumen to viscosity. Lactose content 16.44 per cent; forewarming temperature 96°C. (204.8° F.); storage temperature 30°C. (86° F.); fat 0 per cent*

AGE	NORMAL SKIM MILK	CASEIN REMOVED	CASEIN AND ALBUMEN REMOVED
<i>days</i>	<i>rotation, degrees</i>	<i>rotation, degrees</i>	<i>rotation, degrees</i>
2	18	6	1
9	130	8	1
32	Beyond range	15	1

The proteins, the casein, and the albumen are in a different category. Their colloidal condition, their well known instability under the action of heat and the rapid changes produced in their structure by the activities of bacteria would lead one to expect them to be of primary importance in determining the viscosity of condensed milk.

On account of the complex nature of the milk constituents and their intimate relation to one another it is impossible to separate them in such a way that the effect of each can be determined accurately, but some indication may be obtained by experiments similar to that shown in table 8. A lot of skim milk was divided into three portions, one of which was condensed without further alteration. The two remaining portions were acidified, the

precipitated casein removed by filtration, and the reaction of the filtrate corrected. One of those containing the albumen, lactose, and salts was evaporated. The albumen was removed from the third portion by adjusting the reaction and heating. All three were forewarmed to 96°C., and since they could not very well be brought to an equal concentration of total solids they were evaporated to a uniform lactose content of 16.44 per cent. The cane-sugar concentration of the finished milk was 60 per cent in each case. The original milk increased in viscosity very rapidly; the portion from which the casein had been removed, much more slowly; and the one containing only lactose and salts remained at the initial very low viscosity.

TABLE 9

*Relation of casein and albumen to viscosity. Fat removed; lactose 16.3 per cent; forewarming temperature 63°C. (145.4° F.); storage temperature 30°C. (86° F.)*

AGE	NORMAL SKIM MILK	CASEIN REMOVED	CASEIN AND ALBUMEN REMOVED, CASEIN REPLACED
<i>days</i>	<i>rotation, degrees</i>	<i>rotation, degrees</i>	<i>rotation, degrees</i>
2	3.0	1	1
9	2.5	1	1
30	4.5	1	8
74	37.0	1	130

Similar results were obtained by a variation of this procedure. A lot of skim milk was divided into three parts, one of which was condensed without further change. The casein was removed from the other portions. One of these containing the albumen, sugar, and ash was evaporated. The albumen was removed from the third portion by heat and the casein, redissolved in alkali, was returned giving a milk free from albumen. Sugar was added to each portion to give a 60 per cent solution in the finished milk, and all were evaporated to a uniform lactose content of 16.3 per cent. This experiment differed also from the previous one in that the forewarming temperature was 63°C. (145.4° F.) instead of 96°C. (204.8° F.). The changes in viscosity of these milks are shown in table 9.

The normal milk increased in viscosity slowly, but even at seventy-four days was much less viscous than the highly heated milk of the previous experiment when only nine days old. The very low initial viscosity and lack of change in the milk from which casein was removed indicates that the albumen is a factor only when it has been heated above its coagulating point. The precipitated and redissolved casein shown in the fourth column of table 9 could not be expected to react exactly like casein in normal milk, and we find that while the initial viscosity of the third portion, containing the redissolved casein, was low, it had increased at seventy-four days to 130°. Although this is not exactly comparable with the normal milk, it may be taken as evidence that the casein is the most significant factor in determining the viscosity of sweetened milk. The relation of the albumen to the change in viscosity was also shown by varying the albumen content of the milk by the addition of egg white. The chemical and physical nature of this material is so nearly identical with the lactalbumen of the milk that there is little doubt that it would have the same effect on the viscosity.

When the forewarming temperature was above 90°C. (194° F.) the initial viscosity and the increased viscosity at various storage periods were in direct relation to the amount of albumen added. On the other hand when the forewarming temperature was held below 60°C. (140° F.) the initial viscosity was the same for all lots and the changes, which were small, had no relation to the albumen content of the milk.

The temperature of the pan under ordinary circumstances is too low to affect the albumen or the casein. There is a possibility that the layer of milk in immediate contact with the coil may be momentarily heated considerably above the temperature indicated by the thermometer. A resistance thermometer made especially for this purpose and held so that it was in close contact with the surface of the coil did not give readings appreciably higher than the mercury thermometer projecting through the wall of the pan.

More positive results were obtained by arranging the coils so that they could be heated by circulating water through them at

any desired temperature. Even when the water was held as low as 70°C. (158° F.), the viscosity did not differ from that portion of the milk made in the usual way with steam heated coils. The long exposure to heat which was made necessary by the low temperature of the coils did not affect the viscosity.

#### THE INFLUENCE OF THE ASH CONSTITUENTS

The ash, of course, must not be considered as so much mineral matter in solution in the milk, but as it exists in combination with other constituents of the milk, particularly the casein. It is well known that a certain part of the calcium and the phosphorus exist in the milk in some kind of combination with the casein, and that the stability of the casein is dependent to some extent on this combination.

Sommer and Hart (2) have shown that the temperature at which milk coagulates is controlled, in some degree at least, by the interrelation of the ash constituents. It is also well known that the relation between the casein and its mineral ingredients are more or less disturbed by heat. It is not unreasonable, therefore, to suppose that variations in the ash constituents would have some influence on the viscosity of the concentrated milk.

On account of the difficulties in the way of removing any part of the ash from the milk, it is practicable to vary the mineral matter only by the addition of the separate salts. The combination existing between the ash constituents and the casein and between the ash constituents themselves is still a matter of speculation, and it is obvious that a change in the composition of the ash made in this way cannot be exactly comparable to variations occurring normally.

When one of the normal mineral salts of the milk is added to milk we are unable to say definitely what rearrangements it may bring about, but its effect on the behavior of the milk when exposed to different physical reactions may be taken as an indication of the effect of similar variations occurring normally. According to Van Slyke and Bosworth, (3) normal milk con-



tains 0.274 per cent of citrates in the form of the calcium and potassium salts. On this basis 3500 grams of skim milk, which was the standard batch used in our experimental work, should contain approximately 9.9 grams of total citrates. The addition to 3500 grams of milk, of citrates in the form of calcium citrate in amounts up to 2.8 grams had no appreciable effect on the viscosity when the milk was held at 30°C. (86° F.) for 58 days. This represents an increase in the total citrates of about 28 per cent.

Somewhat different results were obtained by the addition of phosphates. The effect of the addition of molecular quantities of the monobasic and dibasic potassium phosphates is shown

TABLE 10

*Effect of variation in phosphates. Milk solids 26 per cent; fat 0 per cent; forewarming 63°C. (145.4° F.)*

PHOSPHATES ADDED	BEFORE CON- DENSING	AFTER CON- DENSING	VISCOSITY				
	pH	pH.	1 day rotation,* degrees	10 days rotation,* degrees	18 days rotation,* degrees	29 days rotation,* degrees	86 days rotation,* degrees
7 cc. $\frac{m}{1}$ $\text{KH}_2\text{PO}_4$	6.45	6.15	3.9	5.2	5.5	6.2	20.0
7 cc. $\frac{m}{1}$ $\text{K}_2\text{HPO}_4$	6.56	6.24	3.3	4.3	4.8	5.0	33.0
None	6.44	6.21	3.0	3.1	3.2	3.35	18.5

\* Scale.

in table 10. The hydrogen ion determinations, made both before and after condensing, show that these additions had only slight effect on the acidity. The acidity of the milk was distinctly reduced by the addition of the tribasic salt, but this batch was lost. In any case the change in hydrogen ion concentration would be within the limits which we have found to be without effect on the viscosity.

The check milk to which no salt was added was lower in viscosity than any of the others, but the difference was so slight that it can hardly be considered significant. The degrees given in this table are scale readings and indicate a low viscosity even

at eight-six days. The monobasic salt added represents, on the basis of Van Slyke and Bosworth's calculations, an increase in the total phosphates in the milk of about 5 per cent. In another experiment the results of which are given in table 11, slightly larger amounts of the three salts were added. In this case there was no significant difference in the initial viscosity or at eleven days, but at twenty-three days and particularly at forty-four days the three lots to which phosphates were added were decidedly more viscous than the check lot. It should be noted that this is equally true of the monobasic salt which increases the acidity and of the tribasic salt which decreases it. Similar but more marked results were obtained when still larger amounts of salt were added.

TABLE 11

*Influence of phosphates on viscosity. Milk solids, 26 per cent; fats 0 per cent; forewarming 63°C. (145.4° F.)*

PHOSPHATES ADDED	$\frac{m}{l}$	GRAMS	INCREASE IN TOTAL PHOS- PHATES	VISCOSITY			
				1 day	11 days	23 days	44 days
	cc.		per cent	rotation, degrees	rotation, degrees	rotation, degrees	rotation, degrees
KH <sub>2</sub> PO <sub>4</sub> .....	10	1.36	7.3	1.4	2.0	56	140
K <sub>2</sub> HPO <sub>4</sub> .....	10	1.74	9.4	1.5	2.0	9	100
K <sub>3</sub> PO <sub>4</sub> .....	10	2.12	11.4	1.8	2.6	13	120
None.....				1.5	1.7	2	15

#### THE INFLUENCE OF FOREWARMING TEMPERATURES ON THE SEPARATION OF FAT

Nearly all of the experimental condensed milks mentioned in this paper were made from skim milk, and consequently the separation of the fat was not a factor to be considered. It was noticed, however, that in some lots made from whole milk there was a tendency for the fat to separate. In evaporated milk, before homogenization came into general use, it was considered necessary to heat the milk high enough to produce a soft curd, not only to give the milk a satisfactory "body," but also to make it sufficiently viscous to hold the fat in suspension. The viscosity of sweetened milk is usually high enough to accomplish

this purpose, but if the forewarming temperature is low there is a possibility that an objectionable separation of fat may occur.

A comparison of the effect of high and low forewarming temperatures was made by dividing a lot of milk into two portions, one of which was heated to 95°C. (203° F.) by the jacket, while the other part was held thirty minutes at 63°C. (145.4° F.). After condensing in the laboratory pan half of the latter batch was homogenized at 3000 pounds pressure. The results are given in table 12.

TABLE 12

*Influence of forewarming temperature on separation of fat. Total milk solids 28 per cent; sugar solution 60 per cent; storage temperature 30°C. (86° F.)*

FOREWARMING TEMPERATURE	HOMOGENIZING PRESSURE	VISCOSITY				SEPARATION OF FAT, AFTER 29 DAYS
		1 day	8 days	15 days	29 days	
	pounds	rotation, degrees	rotation, degrees	rotation, degrees	rotation, degrees	
63°C. (145.4° F.).....	Not homogenized	3.0	4	7	14.5	Slight
63°C. (145.4° F.).....	3000	7.0	12	16	20.0	Slight
95°C. (203° F.).....	Not homogenized	9.5	26	37	47.0	None(?)

In the lot forewarmed at 95°C. there was at the most only a very slight separation of fat. In the two lots forewarmed at 63°C. there was a noticeable but not objectionable separation of fat. This was slightly less noticeable in the homogenized half.

In another experiment made in the same way the viscosity of the low forewarmed milk was exceptionally low, and there was a decided separation of the fat. This did not occur in the homogenized half nor in the lot forewarmed at a high temperature.

#### SUMMARY

Acidity within reasonable limits, contained air, and the salts of copper have no significant influence on the increase of viscosity of sweetened milk. Cane sugar increases the initial viscosity slightly, but affects the increase in viscosity only as it inhibits the growth of bacteria.

The increase in viscosity is more rapid at higher storage temperatures, but this increase is not in direct relation to the temperature. The effect of the temperature is very slight at 20°C. (68° F.) or lower, but is marked at 30°C. (86° F.) or higher.

The tendency to thicken on standing increases with, but not in direct relation to, the concentration of solids not fat.

The casein is the constituent principally concerned in the production of the viscosity. The albumen is a factor when the forewarming temperature is much above its coagulating point.

The phosphates, probably through their combination with the casein, may have influence on the change in the viscosity. An increase in the total phosphates of the raw milk by as much as 7 per cent will cause a distinct acceleration in the progressive increase in viscosity.

The tendency of the milk to thicken is greatly increased by the high temperature ordinarily used in the forewarming. Satisfactory results from a bacteriological standpoint may be obtained to about 63°C. (145.4° F.) for twenty to thirty minutes. The viscosity of the product made in this way is low, and the tendency for the viscosity to increase in storage is much reduced. When the viscosity is very low in condensed milk made with low forewarming temperature, there is a tendency for the fat to separate. It is not probable that this condition would occur in large batches of milk made under commercial conditions.

#### REFERENCES

- (1) WOLFGANG, PAULI 1913 The viscosity and electrochemistry of protein solutions. *Trans. of Faraday Soc.*, ix, pts. 1-2, 54-56.
- (2) SOMMER, H. H., AND HART, E. B. 1919 The heat coagulation of milk. *In Jour. Biol. Chem.*, xl, no. 1, 137-151.
- (3) VAN SLYKE, L. L., AND BOSWORTH, A. W. 1914 Condition of casein and salts in milk. *N. Y. Agr. Expt. Sta., Tech. Bul. no. 39.*

# BITTERNESS IN EVAPORATED MILK

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In the fall of 1919 a prominent milk condensory experienced considerable difficulty with evaporated milk becoming bitter. This bitterness did not occur in all cans, though made from the same batch of milk sterilized and treated uniformly. The physical appearance of the evaporated milk was normal in every respect, the characteristic bitterness was the only objectionable feature. Some preliminary work was done with the bitter milk, and it was found that ordinary chemical tests failed to reveal any cause for this condition. Plate cultures were made of this bitter milk which showed the presence of an organism. Sterilized sweet milk was inoculated with this organism and it also became bitter. From the appearance of the plate culture there seemed to be present only one type of organism, and this was later found to be correct.

## OBJECT OF THE EXPERIMENT

The object of the following experimental work was to identify this organism, to determine its thermal death point and its proteolytic action on milk proteins, and to make a study of the enzymes produced by it.

## EXPERIMENTAL

### *1. Bacteriological study*

A number of cultures of this organism have been studied both morphologically and culturally. The following is a general description of the organism.<sup>2</sup>

<sup>1</sup> Acknowledgment is due to E. G. Sieveking, Student Assistant.

<sup>2</sup> Credit is here given to I. L. Baldwin of the Biology Department for the description of this organism.

*Morphology.* Form: The organism is definitely rod shaped with rounded ends. When freshly isolated from milk it shows definite capsules which stain readily with gentian violet. Upon continued growth on artificial media it tends to lose its property of forming definite capsules

Size: About 0.4 by 2 microns are the average dimensions of the organism, with relatively little variation in the size of the individual cells.

Motility: No motility has been observed.

Staining properties: The organism stains very readily with the ordinary anilin dyes and is Gram positive.

Spore formation: Spores are formed readily in about forty-eight to seventy-two hours. They are usually formed near the center of the rod and are almost as large as the cell. Definite rims of protoplasm are usually retained around the spores.

*Cultural characteristics.* Agar colonies: Medium sized, elevated, glistening, viscous colonies, showing little tendency to spread were formed rather quickly. In the course of three or four days they lose their glistening appearance owing to the formation of a scum over the surface. Later the colonies seem to dry out leaving only the dry wrinkled scum, which is easily scraped from the agar

Agar slant: The growth resembles that of the agar colonies very closely. At first it is elevated, glistening and viscous and later becomes dry, grayish and wrinkled.

Agar stab: There is a small slightly spreading growth on top with slight granular beaded growth along the line of inoculation.

Gelatin colonies: Small glistening colonies are formed in cup-like depressions, due to the rapid liquefaction of the gelatin.

Gelatin stab: Liquefaction is very rapid, being at first funnel shaped. Complete liquefaction with the formation of a heavy scum occurs later.

Bouillon: A tenacious scum is formed. Slight turbidity is noticed at first, but later the media settles clear.

Potato streak: Growth is at first glistening, elevated and viscous, later becoming dry, granular and wrinkled.

Litmus milk: The milk is peptonized rather quickly, leaving a clear liquid with little change of acidity.

*Fermentation tubes.* Dextrose: No gas is formed but an acid reaction is produced. A heavy scum is formed at the surface with no growth in the closed arm of the tube.

Saccharose: The growth and reaction is the same as with dextrose.

Lactose: The characteristics of growth are the same as with dextrose and saccharose, but very little or no acidity is produced.

The description of this organism agrees in practically every respect with the *Bacillus Panis*, Migula 1900 described by Lawrence and Laubach (1).

### *2. The effect of heat on the organism*

In order to study the resistance of this organism towards heat, sterile tubes of fresh skim milk were inoculated with a pure culture of the organism. These were then incubated until active with spore formation as evidenced by a suspension under the microscope. A set of tubes were then autoclaved for 3, 5, 8, 10 and 15 minutes respectively at 15 pounds pressure, (250° F.). All conditions, such as reaching and maintaining the necessary temperature, and treatment after the expiration of the time limit, were made as uniform as possible. Each tube was plated in triplicate on agar media both before and after autoclaving.

The following table shows the results obtained for the respective time intervals.

TABLE 1

BEFORE AUTOCLAVING	TIME	TEMPERATURE	AFTER AUTOCLAVING
<i>colonies per cc.</i>	<i>minutes</i>	<i>°F.</i>	<i>colonies per cc.</i>
25,200,000	3	250	42,000
22,000,000	5	250	14,500
28,700,000	8	250	560
27,500,000	10	250	None
33,400,000	15	250	None

### *3. Chemical study*

In the study of the chemical action produced by this organism on milk proteins we were chiefly concerned with the cleavage products produced. By the use of different precipitants it is

possible to separate the complex from the less complex hydrolyzed products of protein degradation into reasonably well defined fractions and if the operation is conducted under like conditions these fractions must give a reasonably accurate and consistent result of the progress of the chemical changes. The fractions which seemed the most important were, the production of ammonia, peptones and the less complex protein units, the amino acids. By the use of saturated solution of zinc sulphate and phosphotungstic acid as precipitants, peptones and amino acids can be estimated.

#### METHOD OF PROCEDURE

Lots of 250 cc. sterilized milk were inoculated with the organism and incubated at 37°C. for 15, 30 and 45 days. After the respective periods of incubation 50 cc. of the media was aerated according to the Folin method for the determination of ammonia. After aerating for eight hours the liquid was transferred to flasks and diluted to 100 cc. From this dilution aliquots of 20 cc. were transferred to beakers, neutralized with sulphuric acid and the proteins precipitated with saturated solution of zinc sulphate and phosphotungstic acid.

*Precipitation with zinc sulphate.* To 20 cc. of the above dilution sufficient dilute sulphuric acid was added to make it distinctly acid to methyl red. The solution was slightly warmed and chemically pure zinc sulphate added to saturation. A portion of the zinc sulphate crystallized when the solution was cooled to room temperature. After standing for twenty-four hours the solution was filtered and the nitrogen determined in the filtrate representing nitrogen not precipitated by zinc sulphate.

*Precipitation with phosphotungstic acid.* To 20 cc. of the neutralized solution sufficient sulphuric acid was added to bring the acidity to 5 per cent. To this 20 cc. of a 5 per cent phosphotungstic acid solution was added and after standing for twenty-four hours the solution was filtered and the nitrogen determined in the filtrate, representing the nitrogen not precipitated by phosphotungstic acid.

*Acidity.* The acidity was determined by titrating 20 cc. of the medium with N/10 NaOH and expressed as lactic acid.



*Ammonia.* The ammonia was determined by the Folin aeration method and expressed as ammonia nitrogen.

Table 2 shows the chemical changes brought about by this organism when grown in sterile milk. The results for the nitrogen fraction are based on the total nitrogen present in the milk and the acidity expressed as lactic acid.

TABLE 2

*Showing the acidity, ammonia nitrogen, nitrogen not precipitated by phosphotungstic acid and saturated solution of zinc sulphate*

INCUBATION	ACIDITY	AMMONIA NITROGEN	NITROGEN NOT PRECIPITATED BY ZINC SUL- PHATE	NITROGEN NOT PRECIPITATED BY PHOSPHO- TUNGSTIC ACID	NITROGEN AS PEPTONES
<i>days</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
15	0.297	4.56	39.67	20.76	18.71
30	0.517	5.44	46.15	26.40	19.73
45	0.540	5.53	46.59	25.75	20.74
Blank	0.215	0.48	1.84	0.92	0.92

#### ACTION OF THE ENZYMES

The nature of the organism, and its activity on protein suggested the possibility of the presence of proteolytic enzyme or enzymes. It was the aim to study the secreted enzymes or exoenzymes which, owing to their filterability may be separated from foreign matter and obtained in an active state.

Flasks containing 250 cc. of sterile milk were inoculated with the organism and incubated at 37°C. for thirty-three and sixty days. At the end of the respective periods of incubation, a portion of the liquid was filtered through a Berkfeld filter. To prove the absence of organisms or spores plate cultures were made. Only in a few instances was there any contamination.

To test the activity of the enzymes obtained from the Berkfeld filter a 2 per cent solution of casein and egg albumin was prepared as follows: 2 grams each of casein and egg-albumin were added separately to 100 cc. of distilled water and sterilized. Plate cultures were here also made to prove the absence of organisms. To each flask containing the sterile proteins 10 cc. of the filtrate, obtained from the media were added and digested from

three to six days at a temperature of 37°C. Digestion was made in neutral (phenolphthalein), 0.2 per cent acid (hydrochloric acid) and 0.2 per cent basic (sodium carbonate) solution.

TABLE 3

*Showing the action of the enzymes on casein, the nitrogen not precipitated by zinc sulphate, phosphotungstic acid and the peptones*

INCUBATION	DIGESTION	REACTION	NITROGEN NOT PRECIPITATED BY ZINC SULPHATE	NITROGEN NOT PRECIPITATED BY PHOSPHOTUNGSTIC ACID	NITROGEN AS PEPTONES
<i>days</i>	<i>days</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
33	3	Neutral	60.75	25.35	35.40
33	3	0.2 per cent acid	16.55	5.10	11.45
33	3	0.2 per cent basic	38.85	14.00	24.85
60	3	Neutral	62.5	31.7	30.80
60	3	0.2 per cent acid	20.00	7.6	12.40
60	3	0.2 per cent basic	37.20	9.85	27.35
60	6	Neutral	89.65	47.85	41.80
60	6	0.2 per cent acid	16.75	8.00	8.75
60	6	0.2 per cent basic	78.75	40.10	38.65

TABLE 4

*Showing the action of the enzymes on egg albumen, the nitrogen not precipitated by zinc sulphate, phosphotungstic acid and peptones*

INCUBATION	DIGESTION	REACTION	NITROGEN NOT PRECIPITATED BY ZINC SULPHATE	NITROGEN NOT PRECIPITATED BY PHOSPHOTUNGSTIC ACID	NITROGEN AS PEPTONES
<i>days</i>	<i>days</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
33	3	Neutral	44.30	15.80	28.50
33	3	0.2 per cent acid	19.00	6.85	12.15
33	3	0.2 per cent basic	34.10	15.15	18.95
60	3	Neutral	50.10	24.50	25.60
60	3	0.2 per cent acid	27.45	10.10	17.35
60	3	0.2 per cent basic	31.75	10.20	21.55
60	6	Neutral	81.50	46.60	34.90
60	6	0.2 per cent acid	22.70	10.95	11.75
60	6	0.2 per cent basic	48.55	22.55	26.00

At the time expiration the enzyme action was determined by estimating the nitrogen not precipitated by zinc sulphate and phosphotungstic acid.

Tables 3 and 4 show the proteolytic action of the enzyme. The per cent nitrogen is based on total nitrogen.

## DISCUSSION

From table 2 it is seen that this organism produces rapidly a large quantity of peptones and lower complex nitrogenous compounds. The excessive peptonizing function of this organism may be the primary cause of the bitterness. It is not impossible that the cleavage products produced by this organism differ in their chemical aggregation from that produced by other active peptonizing organism. This bitterness in evaporated milk has been observed by other investigators, namely, O. F. Hunziker (Condensed Milk, 1st edition, pp. 172-174) and more recently by B. W. Hammer (Studies in Abnormal Evaporated Milk 1919). The increase in acidity during the period of fifteen days incubation was very slight, and this is in agreement with that condition found in the bitter evaporated milk. The production of ammonia increased during the period of incubation from 0.48 to 5.53 per cent. This increase also emphasizes the proteolytic activity of this organism, which by some investigators is considered an index in determining the rate and degree of protein katabolism.

## SUMMARY

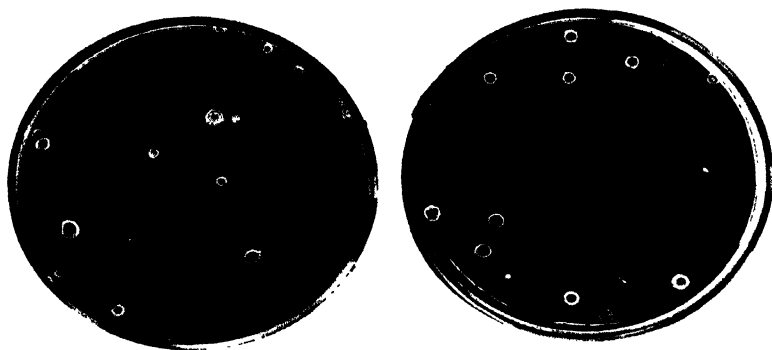
1 From the results of the bacteriological study the organism agrees morphologically and culturally with *Bacillus panis migula*, described by Lawrence and Laubach.

2. The thermal death point of this organism lies between eight to ten minutes exposure to 250° F. under steam pressure.

3. The substance secreted by this organism is proteolytically active, producing both peptones and amino acids. The results of the action on neutral, acid and basic substrate indicate the presence of more than one specific enzyme.

## REFERENCES

- (1) LAWRENCE AND LAUBACH; Studies on aerobic spore-bearing non-pathogenic bacteria. Jour. Bacteriol, i, 493.



PHOTOGRAPH OF THE ORGANISM STUDIED, FROM A SIXTY-HOUR PLATE CULTURE

# THE EFFECT OF PASTEURIZATION ON THE NUMBER OF BACTERIA IN MILK WHEN THIS IS DETERMINED BY THE DIRECT MICROSCOPIC COUNT<sup>1</sup>

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It has been pointed out in a previous paper (1) that in the examination of milk it is desirable to obtain such information as will permit the analyst to determine as far as possible the conditions under which the milk was produced, and also to judge of its future, or keeping quality. It is obvious that in the case of pasteurized milk, any method which involves the growth of bacteria can give no indication concerning the history of the milk previous to heating. It has been thought that the direct microscopic determination of the number of bacteria did not possess the above limitation and could therefore be used more successfully in the examination of pasteurized milk than could any other method.

It is a well known fact that many bacteria are so changed on heating as not to be detected in the microscopic examination of stained preparations. The use as a culture medium of milk which contained such a number of bacteria that they were easily demonstrable with the microscope, entails no disadvantages in the subsequent examination of the pure cultures grown therein. In the heating, incident to sterilization, the cells are so changed that they can not be found with the microscope. The magnitude of the effect of lower temperatures such as are used in the pasteurization of market milk is unknown. It has been mentioned by a number of workers that the dead cells do not possess the same staining properties as the living cells, when preparations are made from milk before and after pasteurization. Kufferath (2) in a discussion of the methods of determining bac-

<sup>1</sup> Published with the permission of the Director of the Wisconsin Experiment Station.

teria in milk refers to the examination of stained preparations and states that dead bacteria do not stain, but that their presence is made evident by the fact that they appear in the film as clear areas in the back ground of stained casein, as do the cells in a preparation made with India ink according to the method of Burri. He states that an idea of the number of bacteria present before heating can be obtained by noting the clear areas.

Again it has been assumed by some that the heating does not change the staining properties of the cells and that therefore the direct microscopic method gives the same information in regard to a sample of milk, irrespective of whether it is applied before or after pasteurization. The following statements are taken from an article recently published (3).

A microscopic count of pasteurized milk will show exactly what a similar count would show in raw milk—namely the number of bacteria which have gained access to milk during its production and delivery and the amount of development through growth and multiplication. . . . The direct microscopic method of counting bacteria, revealing the living as well as the dead bacteria, enables us to put pasteurization in its proper place in the scheme of milk control and to proceed with our enforcement of bacterial standards with a success not allowed with the plate count.

For the purpose of determining the effect of pasteurization on the staining properties of bacteria in the direct microscopic method of milk examination, a number of milks were pasteurized in the laboratory at 145° F. for thirty minutes. The milks were cooled at once, and stored at about 50° F. Microscopic preparations were made from the milk immediately before pasteurization, and at varying intervals thereafter. The method of staining was kept uniform throughout the staining of any series of samples.

It is probable that the effect of heating on the number of bacteria losing their staining properties will vary from sample to sample; certain bacteria are probably more affected than others. The tubercle bacillus in broth stains as well after pro-

**TABLE 1**  
*The comparative percentage of the bacteria in raw milk that can be detected with the microscope at varying intervals after pasteurization at 145°F. for thirty minutes*

	SAMPLE															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Raw.....	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Immediately after pasteurization...	60	30	77	30	39	60	25	18	83	24	75	50	43	56	50	69
Six hours after pasteurization...	50	8	111	30	13	37	18	27	21	4	25					
Twenty-four hours after pasteurization.....																
Forty-eight hours after pasteurization.....	25	30	166	100+	100+	94	100+	100+	100+	100+	100+	20	20	11	6	3

longed heating at 212° F. as do the same cultures in which the live cells are present. Since different kinds of bacteria will react differently when the milk in which they are present is heated, no uniformity in results would be obtained in the examination of a series of milks that vary qualitatively in their bacterial content.

In the work here reported market milks were used. The technique of staining was that suggested by Breed. The per-

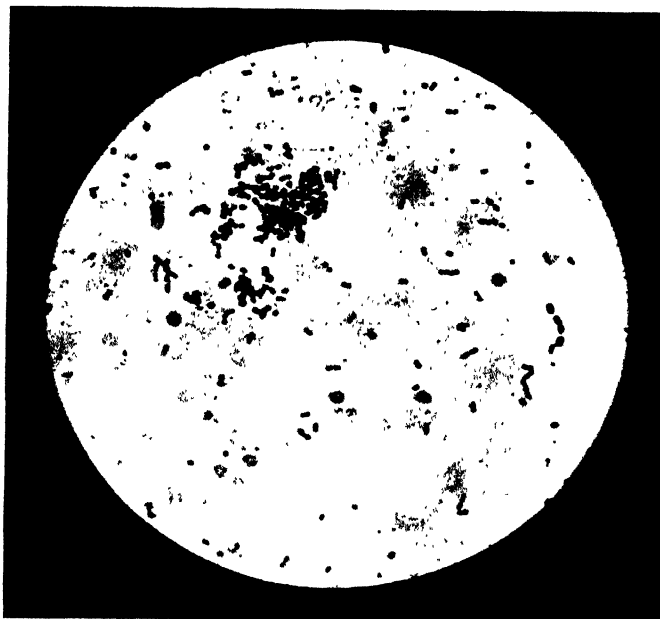


FIG. 1. A PREPARATION FROM RAW MILK

Many lactic acid bacteria are visible.

sonal factor has been eliminated by the fact that a number of individuals, working at different times, were concerned in obtaining the results reported. Approximately a hundred samples of milks, taken at various times of the year, have been examined. A detailed count of the bacteria has been made in a number of samples and the data are reported in table 1. The preparations made from the remainder of the samples were not counted; they



were, however, examined with sufficient care to determine whether the heating had had any effect on the staining of the bacteria. In practically every instance there has been a more or less marked decrease in the number of cells that could be detected. The data given in table 1 are to be considered as typical of all. For purposes of comparison the number of cells per unit volume of the raw milk is expressed as 100, the number found in the examinations made after the pasteurization, as percentages of 100.

From table 1 it is to be noted that the percentage reduction varies from sample to sample. Sample 3 in no examination revealed less than 77 per cent as many cells as were present in the raw milk; and in sample 16 only 3 per cent as many bacteria were found twenty-four hours after pasteurization as were noted in the raw milk. It is believed that these percentages represent the probable limits of the effect of the pasteurizing temperature on the number of bacteria in milk as shown by the direct microscopic method. Thirteen out of the sixteen samples showed between 10 and 30 per cent as many bacteria after pasteurization as in the raw milk, and these reductions probably represent the average that will be noted in practice. In two out of the 16 samples reported in table 1, the minimum number of bacteria was found immediately after pasteurization; in six samples the minimum number, at the sixth hour; in five, at the twenty-fourth hour; and in three, at the forty-eighth hour.

The bacteria that have not been killed in pasteurization will begin to grow when the milk is stored, and sooner or later, the number of cells in the pasteurized milk will exceed the number in the raw milk. The rapidity with which this will occur will depend on the temperature of storage and the kind of bacteria.

In table 1 the expression 100+ signifies that growth had occurred and the number of bacteria was much in excess of that in the raw milk.

Pasteurization of milk inoculated with pure cultures gave results similar to those with naturally inoculated milks. The procedure for this was as follows: Portions of fresh whole milk of a low bacterial content were inoculated with a pure culture of

the desired organism, which had been previously cultivated in sterilized milk, with frequent transfers, in order to become accommodated to this medium. These milk cultures were seeded separately into raw milk in such amounts that the bacteria were fairly numerous on a Breed plate. Stained prepara-

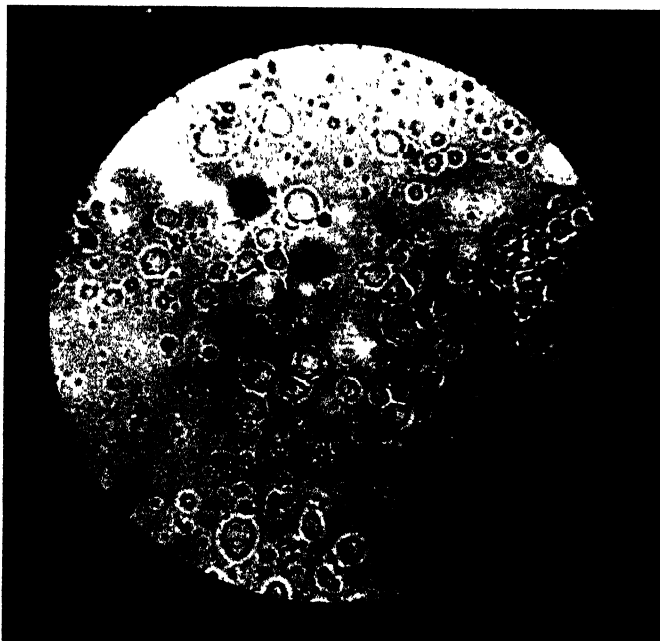


FIG. 2. A PREPARATION MADE FROM THE SAME MILK, PASTEURIZED

The milk had been stored at 50° F. for 24 hours after pasteurization when this preparation was made. The photograph shows fewer bacteria than was the actual case, as the faintly stained cells did not give sufficient contrast to be photographed.

tions were made immediately from these milks, after which they were pasteurized, cooled, and stained again in the same manner. They were then stored in the ice box, and preparations were made again at the end of twenty-four and forty-eight hours. The results are listed as follows:

<i>Name of organism</i>	<i>After 24 hours</i>
<i>B. coli</i> .....	No apparent change in staining or in number of cells
<i>Staphylococcus pyogenes aureus</i> .....	No apparent change in staining or in number of cells
<i>Streptococcus pyogenes</i> .	Cells unevenly stained—some very faint—decrease in number
<i>Bact. lactis acidi</i> (1) ..	Cells unevenly and faintly stained—marked decrease in number
<i>Bact. lactis acidi</i> (2)....	Cells unevenly and faintly stained—marked decrease in number
<i>Micrococcus</i> . . . . .	Cells faintly stained—decrease in number
<i>Bact. bulgaricum</i> .. ..	Cells faintly stained—no apparent decrease in number
<i>B. anthracis</i> .....	Only an occasional cell—only faintly stained
<i>B. subtilis</i> .....	No cells stained

Detailed counts were not made of the stained preparations of these cultures except of that of the micrococcus culture, and this was done because it could not be definitely told from observations whether there had been a decrease or not. The cells of this organism are unusually large and because of their size, although stained very faintly, could be counted, whereas a smaller cell would probably not have been detected. However, only 75 per cent as many cells could be found in the preparations made from the heated milk cultures as were present in the unheated. The most decided decrease was noted with *B. subtilis* and *B. anthracis*. No cells at all took the stain in the *subtilis* preparation, and only an occasional one, in the anthrax, after twenty-four hours storage; and indications of this disintegration of these cells were evident in the preparations made immediately after pasteurization, where the failure to take the stain but poorly was very noticeable. Stained preparations of the other cultures showed about the same average decrease as was noted with the market milks. While it is reported above that no apparent decrease was obtained with *B. coli* and *Staphylococcus pyogenes aureus*, it is possible that had these preparations been subjected to a careful count, a decrease might have been detected.

The observation of Kufferath, that the dead bacteria in heated milk appear as clear areas against a stained background was never noted in any of the numerous preparations which were made of heated milks, either of a high bacterial content or of milks which had been inoculated with pure cultures of representative milk organisms. The degree to which the bacteria vary in their staining properties after heating was from a normal color to that which was barely perceptible, which differences no doubt were caused by the type of organisms present. Staining within these limits even showed considerable variation; some of the cells were faintly but evenly stained, some showed decidedly uneven staining, especially noticeable in the chains and clumps of cells, but when no stain at all was taken this was evidenced only by a decrease in number and never by unstained areas in the shape of bacterial cells.

The conclusion that must be drawn from the data presented, and which is confirmed by the results not here reported is that a direct microscopic count of bacteria in pasteurized milk gives a very imperfect picture of the bacterial content of the same milk before pasteurization, the number of bacteria revealed may not be over 3 per cent of the number in the raw milk.

#### REFERENCES

- (1) HASTINGS, E. G.: The comparative value of quantitative and qualitative bacteriological methods as applied to milk, with especial consideration of the methylene blue reduction test. *Jour. Dairy Sci.*, ii, 293-311, 1919.
- (2) KUFFERATH, H.: Le control bactériologique et hygiénique des laits. *Methods employées et appréciation des résultats. Ann. de l'Inst. Pasteur*, 1919, xxxiii, 462-483.
- (3) WERNER, PERCY: Microscopic count of bacteria. Its value in grading pasteurized milk. *Hoard's Dairyman*, May 7, 1920.

# IS ROPY MILK BECOMING A MORE SERIOUS DAIRY TROUBLE

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Ropy milk is an old dairy trouble, and outbreaks of it are brought to the attention of the Dairy Department every year. There seems to be some basis for the feeling that this trouble is becoming more common, and this was emphasized the past season by encountering an outbreak which involved more than a hundred farms and presented the general picture of an epidemic. As both the causal organism and the epidemic nature of this outbreak were somewhat unusual, it seemed desirable to bring the matter to the attention of the dairy industry.

In presenting the details of this epidemic, the authors have drawn freely upon the observations and experiences of men in the milk industry, particularly upon certain of these men who are well trained in dairy bacteriology. It would be a pleasure to make more detailed and specific acknowledgment of these contributions were it not for the expressed wish of the commercial firms involved that no mention of their companies or of their men appear in the publication.

While both authors were located at the New York Agricultural Experiment Station, they made fairly extensive observations in connection with outbreaks of ropy milk; isolating and studying the causal organism in a number of cases. Through the kindness of the Director, W. H. Jordan, and Dr. R. S. Breed, the notes of these observations and some made by Mr. G. L. Ruehle have been placed at our disposal, and have been used in connection with this article. Grateful acknowledgment of this kindness is gladly given.

## WHAT IS ROPY MILK

The term ropy or slimy is applied to milk which has become noticeably more viscous than ordinary milk. When this ropiness is only slightly developed, it is ordinarily overlooked. It is

sometimes noticed from the fact that the milk pours more slowly, or because the last portion drips from the container with the formation of an evident thread of milk.

Where the viscosity is more pronounced, the milk may be drawn out by means of a fork or similar object into threads. In extreme cases these threads may be fine and silky, and more than a foot long, though more frequently they break when only an inch or less in length.

In extreme cases, the milk takes on almost the consistency of a sticky, stiff dough, and a cup of it may be inverted without the milk being spilled.

Ropy milk should not be confused with the results of garget. Garget is an inflamed condition of the udder of the cow, and milk as it comes from such inflamed udders frequently contains white masses or strings of coagulated material. Milk which becomes ropy, on the other hand, comes from healthy udders and is normal when drawn, but the ropiness may appear at any time after twelve hours.

There are at least three common and distinct types of ropy milk: (1) the ropiness produced in milk drinks by the Bulgarian bacillus, (2) the ropiness in starter for butter making, resulting from the degeneration of the starter culture, and (3) the ropiness which appears in sweet milk.

The ropiness with which this publication is primarily concerned makes trouble in connection with city milk. It appears in sweet, well cared for milk, and ordinarily as soon as the milk begins to sour, the viscosity disappears. Moreover, this ropiness in sweet milk develops most markedly at the surface in contact with the air, and rarely appears unless the milk is kept relatively cold. When the milk is held at higher temperatures, acid develops and destroys the viscosity.

#### WHY IS ROPY MILK OBJECTIONABLE

With milk, as with other foods, custom is a large factor in establishing market demands. There is no question but that our retail milk trade calls for a sweet milk of normal consistency and taste. While the growth of the ropy milk germs ordinarily

produces little change in the taste of the milk, these organisms do change its consistency so noticeably as to arouse the suspicions of the consumer.

The agitation of recent years for a milk of low germ content has made the consumer very suspicious of any evidence of germ growth in milk, and doubly so of unusual appearances.

While there is no evidence nor any reason to believe that the growth of the ropy milk organisms is in any way harmful to the consumer, such milk is not acceptable to the American consuming public, and accordingly is neither profitable nor desirable in the city milk supply.

#### HISTORICAL

Among nomads, milk is either consumed at once, or is soured so promptly that there is little opportunity for ropiness to appear in the sweet milk. When dairymen adopted a fixed abode and milk soured more slowly, there was a better chance to observe ropiness. There is naturally no record of its first appearance, but the wide-spread use of sweet, curdled, viscous milk by the Scandinavians, the use of viscous whey in cheese making by the Hollanders, and the prevalence of viscous, sour milk beverages among the Turks, Armenians, and their neighbors, suggest that next to the souring of milk, its increase in viscosity is its most common change.

The earliest attempts at a scientific explanation of this ropiness were made by chemists but probably the first observations of the connection between germ life and these changes in milk were made by Lister (1) in 1873. Another early observation of the connection between germ life and the production of ropy milk is that of A. Schmidt (2) in 1883. His reference to this dairy trouble suggests that in Germany it was then well known, and was the occasion of considerable economic loss. While the work of Schmidt did not include the isolation and study of the causal organism, his tests of the readiness with which the ropiness was transferred to sound milk by the addition of a few drops of the ropy milk, and later likewise transferred from this to a second sample of sound milk, made plain the contagious nature of the trouble.

In 1889 there appeared at short intervals in the *Milch Zeitung* an article by Adametz (3) describing *Bacillus lactis viscosus* as the cause of ropiness in sweet milk, and one by Weigmann (4) describing, but not naming, the organism causing viscosity in sour whey. A number of organisms have been isolated, considered as new, and named in connection with the development of ropiness in milk and dairy products. Of the outbreaks in city milk supplies in this country studied by Marshall (5), Ward (6), Cole and Hadley (7), Buchanan and Hammer (8), and Harding and Prucha (9), the causal organisms seem to be identical with, or closely related to *Bacillus lactis viscosus*, Adametz. The points at which organisms producing ropy milk most commonly differ from the original description of *Bacillus lactis viscosus* are in the matter of acid and gas formation. The observations at hand suggest that if a sufficiently large number of cultures were examined, it might be possible to arrange a fairly continuous series of cultures, otherwise quite similar, but differing in that at one extreme the milk becomes slightly alkaline, while at the other the milk becomes distinctly acid. This is all the more striking in view of the fact that the alkali-producing and neutral forms are evidently sharply inhibited in milk by the formation of acid by the ordinary milk flora.

These cultures quite generally attack milk sugar and some other sugars, and in some cases appreciable quantities of gas are produced. Apparently this group of ropy milk organisms offers good material for a critical study of the validity of acid and gas formation as a basis for the separation of species.

#### THE EPIDEMIC

About the middle of June, 1919, complaints of ropy milk from customers of a large dairy company led to observations at the bottling plant handling this portion of its supply. Ropy milk is not an uncommon experience in connection with the city milk trade and when it appears a prompt study of the source of supply usually shows that milk infected with ropy organisms is being furnished by one or two farms. Since more or less of this trouble



appears every season, its treatment has become practically a routine matter. ¶

In the present instance, samples from each patron's milk were collected in well steamed bottles as the cans were being emptied, and these bottles, capped or stoppered with cotton, were held at low temperature and observed for the development of ropiness.

Unless the infection is fairly heavy, the ropiness will rarely be evident before twenty-four hours, and may not appear before forty-eight hours. Where the germs are fairly abundant, the entire upper surface of the cream becomes noticeably viscous. Examinations are best made with a small bent platinum wire which is heated to redness and cooled before the examination of each bottle, so as to prevent the transfer of germs from one bottle to the next. When this wire is thrust below the surface of the cream and is withdrawn, the viscous milk is drawn out into a thread the length and thickness of which vary with the degree of ropiness. In making such examinations of milk in bottles which have been stoppered with cotton, care should be exercised not to be misled by the presence in the milk of cotton fibers. When such a fiber is caught by the wire, it often closely resembles a thread of ropy milk. Where such a wire is lacking, wooden toothpicks or forks may be used. However, unless a fresh one is used for each bottle, examinations on succeeding days may lead to wrong conclusions because of the transfer of germs from one bottle to another.

Where the infection is not abundant, the ropiness of the cream may be confined to small islands or even to very small points on the surface. At these points there is evidently the development of colonies of the germs causing the trouble. The experienced observer can often locate these points of ropiness by observing the presence of what appears to be small drops of fat in the cream. In many cases the milk surrounding these drops of fat is very ropy. On the other hand, in an occasional bottle these drops are evident and the milk is not ropy, while in many cases the milk is ropy without the presence of these drops of fat. While the relation of these drops of fat to the germs causing the trouble is not entirely clear, the connection between these drops and the ropiness is too regular to be a mere accident.

Samples were taken and examined in this way from the milk of each of the 140 patrons bringing milk to the bottling plant in question, and ropiness was noted one or more times in samples from 116 of these farms. Late in the season when a neighboring cheese factory closed and a number of its patrons transferred to the bottling plant, they also were found to be bringing the ropy organisms. Inquiries in the community in a few cases where a cow was kept by a family who was not otherwise connected with the dairy business showed that here, too, they were having trouble with ropy milk. In short it appeared that here was a community-wide epidemic of ropy milk, which not only included the larger part of the patrons of the bottling plant, but also included people not directly connected with the milk shipping industry.

#### OBSERVATIONS ON FARMS

The finding of ropy milk organisms in the milk as delivered at the bottling plant was followed by a collection of samples at the farms.

These samples ordinarily showed the presence of the ropy organisms in the utensils and in many cases also showed that they were present in the water in the cooling tank.

The observations made at one farm are sufficiently important to deserve special mention. At this farm the germs were repeatedly shown to be present on the utensils and in the water of the cooling tank. From the cooling tank the water flowed to the stock watering tank located in the barn yard, and the presence of the ropy milk germs was repeatedly demonstrated in the water from the stock tank.

The stock tank overflowed and leaked into the barn yard forming considerable mud through which the cows came to drink.

In drinking, the cows rubbed against the wet sides of the tank, and their coats also became moistened by the noses of their companions. Some mud also got upon their coats.

On two different occasions, material collected from the flank and udder of these cows and put into sterile milk produced characteristic ropiness indicating the presence of the ropy milk

germs. Both of these tests were made by the bacteriologist of the milk company. At one of the tests, one of the authors (H.) was present and observed the conditions of the barn yard and the details of the test, and he sees no reason to doubt that under these conditions the ropy germs were present on the coat of the cow and from it could have been transferred to the milk.

#### METHOD OF DISTRIBUTION

Finding this ropy milk organism throughout the milk of the community naturally raises the question as to its method of distribution.

The most evident point of contact among the farms supplying milk to the bottling plant is the can-washing vat at the plant. A can bringing milk infected with the ropy milk organism adds to this washing vat its quota of these organisms, some of which will be transferred to the cans subsequently washed in this same solution. As a check on the spread of germs in this way, the cans and covers were regularly passed over steam and drying jets. However, this latter process, as actually carried on, did not produce sterile cans, and the treatment of the can covers was even less efficient. The drying process likewise did not result in dry cans, and the germs surviving the steaming had an opportunity to multiply before the cans were again filled with milk. Under such circumstances it is evident that ropy milk germs might spread among the patrons of the milk plant. On the other hand, such conditions are so common among milk plants as to be fairly typical. Ropy milk appears almost every year in the supply brought to each bottling plant, and this is the first wide-spread distribution of this kind which has been reported, although this milk trouble has been known for at least a century. If the can-washing vat was the avenue of distribution, it is hard to understand why this wide-spread dissemination should be limited to this particular milk plant, unless the conditions at this plant were unusually favorable for such spread, or the organism in question was one of unusual vitality.

Everything which could be found in connection with the plant suggested more than usual care in manipulation, and rather promptly after the appearance of this trouble, the usual steaming process was supplemented by immersing the washed cans and their covers in a strong solution of bleaching powder. Careful tests of the cans after such treatment indicated that they were not then harboring the ropy milk germs. Notwithstanding this careful treatment of the cans, which was continued for some months, the trouble continued to appear among the farms. The possibility of infection at the can-washing vat will in no wise account for the appearance of the ropy milk among the patrons of the cheese factory, nor in the private dairies which were not connected with the cheese factory or the bottling plant.

The organism causing the trouble, while evidently a representative of the *Bacterium aerogenes* group, was not sufficiently different from the forms ordinarily encountered in ropy milk to explain this unusual distribution.

In the absence of an apparent avenue of distribution, the fairly common presence of *Bacterium aerogenes* in water supplies may be significant. The experiences of Adametz led him to stress water supplies as sources of ropy milk germs, and similar views were held by Ward, but in the last two decades attention has apparently drifted away from the possibility of infection through this channel. It is unfortunate that the conditions in connection with this outbreak were such as to preclude a careful study of the water flora and its relation to this trouble, though samples of the water in which the cans of milk were being cooled on farms furnishing ropy milk, in many cases, showed the presence of the ropy milk organisms. It is true that their presence in this water might be explained on the supposition that they were brought into the cooling tanks on the infected cans, but the well known ability of these germs to live in water calls for a more careful study of the relation of the water flora to the spread of ropy milk.

## THE CAUSAL ORGANISM

The germs connected with the different forms of ropy milk have been described in detail by Buchanan and Hammer. The one associated with this outbreak was a short, non-motile, encapsulated rod which did not form spores, but attacked dextrose, lactose, and saccharose with the formation of acid and gas. In milk at 20°C., there was a marked increase in viscosity within twenty-four hours, together with a slow formation of acid which later became sufficient to curdle the milk. As the acid increased, the viscosity decreased. This organism is evidently a member of the *Bacterium aerogenes* group.

While this organism is by no means unknown in connection with this trouble in milk, it is evidently not the ordinary form producing ropiness in sweet milk, since it was found in only one of ten recent outbreaks in the country tributary to Chicago, from which cultures have been studied by the authors, and it occurred only once among eight cultures isolated and studied by the authors between 1904 and 1908 at the New York Agricultural Experiment Station. It should, however, be noted that Eckles is reported to have found representatives of this group fairly common in connection with outbreaks in Missouri (10).

## RESISTANCE TO HEAT

In as much as this outbreak occurred in connection with a milk supply which was regularly pasteurized, the ability of the organism to withstand heat was a matter of considerable importance.

As ordinarily understood, the thermal death point is the temperature at which a twenty-four hour culture grown on agar slope, suspended in bouillon, and filtered through paper to remove masses of culture is completely destroyed on ten minutes exposure. There is some difficulty in accurately measuring the ten-minute exposure, because of the time required for the temperature to pass through the wall of the container, and to heat the bouillon in which the culture is suspended. The first

loss of time can be reduced to a minimum by the use of Sternberg bulbs with a capacity of 8 to 12 cc., which can be blown from glass tubing and sealed while hot. On breaking the capillary end below the surface of the bouillon, 5 to 7 cc. of the latter will be drawn into the bulb. If the capillary tube is again sealed, the bulb containing the culture may be completely submerged. The extremely thin walls offer little resistance to the passage of heat, and the small volume of fluid in the partially filled bulb quickly acquires the desired temperature, particularly if the bulbs are submerged and agitated by a suitable device.

The thermal death point of a culture of *Bacterium aerogenes* (B1) from this outbreak was compared with that of a culture of *Bacillus lactis viscosus*—*Bacterium viscosum*—(N), isolated about eighteen months earlier from an outbreak of ropy milk in the product of another large milk company.

TABLE 1  
*Growth after heating in bouillon to temperature for ten minutes*

CULTURE	50°C.	55°C	60°C.	65°C.
B1	+	+	—	—
N	+	—	—	—

The bulbs containing the cultures were exposed for ten minutes in triplicate at temperatures of 50°, 55°, 60°, and 65°C. After removal from the hot bath, the bulbs were promptly cooled to room temperature, and the contents of each bulb divided among three tubes of sterile skim milk. The tubes of inoculated skim milk were placed at 20°C. and observed for ropiness and other evidences of growth. The results of these observations are given in table 1.

The above results suggest that the culture of *Bacterium aerogenes* (B1) was somewhat more resistant to heat than was the culture of *Bacterium viscosum* (N). In this connection it should be remembered that the former culture had been isolated recently, while the latter had been carried for many months in the laboratory.

Because of the importance of the heat relation of the ropy milk organisms, the thermal death points of a second series of nine cultures were determined according to the method already given.

In this series the culture (B2) was a representative of the *Bacterium aerogenes* group, isolated quite independently from the same outbreak, and evidently a duplicate of B1. The other cultures used in this series were obtained within a few preceding months from different outbreaks of ropy milk within the Chicago territory, except the culture I which came about a year earlier from an outbreak in the University dairy. With the exception of B2, these cultures were apparently all representatives of the *Bacterium viscosum* group

The results of this test are given in table 2.

TABLE 2  
*Growth after heating in bouillon to temperature for ten minutes*

CULTURE	55°C.	60°C.	65°C.
B2	+	—	—
B3	+	—*	—
B4	+	—*	—
B5	+	—	—
B6	+	—*	—
B7	+	—*	—
B8	+	—*	—
B9	+	+	—
I	—	—	—

\* Indicates that one of the three Sternberg bulbs produced growth.

From the results given in table 2 it is seen that five of the nine cultures show a thermal death point at approximately 60°C., since of three bulbs of each culture simultaneously exposed to this temperature for ten minutes two failed to survive, while in the third bulb some of the germs remained alive. In the case of culture B9, germs in all three bulbs survived, while in cultures B2, and in I, all perished during the heating at this temperature.

It is also of interest to note that culture I, which had been cultivated in the laboratory for about a year, showed a distinctly lower thermal death point.

Since the temperature and time of heating used in commercial pasteurization is 140°–145° F. (60°–62.8°C.) for thirty minutes, it is difficult to understand from the above results why these germs made trouble in the pasteurized product of the milk companies.

In the established methods for thermal death point determinations, care is exercised to avoid the presence of masses of growth in the material as tested, and the test is made with bouillon through which heat passes readily. Under commercial conditions the ropy milk germs grow for some hours in the full milk, developing colony masses of growth, and full milk offers decidedly more resistance to the passage of heat than does bouillon.

Accordingly, in order to copy commercial conditions more closely, flasks of sterile whole milk were inoculated from transfers of the same cultures used in the first experiment. After standing about eighteen hours, the milk was decidedly ropy. It was then, without filtering, transferred to Sternberg bulbs, and these bulbs were exposed 10 minutes in triplicate at 55°, 60°, and 65°C. After being heated, the bulbs were cooled, the material transferred to sterile milk, held at 20°C., and observed as in the first experiment.

The results of this test are given in table 3.

TABLE 3  
*Growth after heating in milk to temperature for ten minutes*

CULTURE	55°C.	60°C.	65°C.
B1	+	+	—
N	—	—	—

Again there is evidence that the culture of *Bacterium aerogenes* (B1) was distinctly better able to withstand heating than was the culture of *Bacterium viscosum* (N). In this case the result is in part due to the fact that B1 grew much the better in the milk, and as a result, the milk containing this culture was much more ropy at the time it was heated. Particularly in the milk containing the culture of B1, the development of the ropy germ exceeded anything which would probably occur in milk



about to be pasteurized commercially. It is therefore of interest to note that, while these germs were able to survive a heating for ten minutes at the minimum pasteurizing temperature, they did not survive a heating for ten minutes at 65°C., which is but slightly above the maximum temperature often attained in the commercial pasteurizing process.

In order to more severely test the ability of masses of bacteria to survive high temperatures, a fourth series was tested, using the same cultures. In this case transfers were grown twenty-four hours on agar slopes; the somewhat tough growth was rubbed loose with a platinum wire, and the fragmented material washed out into sterile whole milk, using about 10 cc. of water in making the transfer. The resulting suspension containing considerable masses of bacteria was put, without filtering, into Sternberg bulbs and heated in triplicate at 55°, 60°, and 65°C. These heated bulbs were cooled and tested as in the preceding series. The results are given in table 4.

TABLE 4  
*Growth from bacterial masses after heating to temperature for ten minutes*

CULTURE	55°C.	60°C	65°C.
B1	+	+	+
N	+	-	-

Once more a greater ability to withstand heating is shown by the culture of *Bacterium aerogenes* (B1). However, in this case also, the greater vigor of its growth on the agar slope is undoubtedly a factor in this result.

Comparing the results from this fourth series with those of the two preceding trials, it is seen that the increased size of the bacterial masses in the material tested measurably increases the ability of the germs to withstand brief exposures to high temperatures.

It has been shown that the thermal death point of these cultures of ropy milk organisms is approximately 60°C. (140° F.) on a ten-minute exposure, and that under commercial conditions the resistance of these germs may be measurably increased.

Accordingly, all the cultures were given a test designed to be more severe than the most extreme conditions which would occur in connection with commercial pasteurization.

Flasks of sterile whole milk were inoculated from the various cultures about eighteen hours before the test. At the time this inoculated milk was transferred into the Sternberg bulbs, a number of the flasks were extremely ropy, and some ropiness was evident in all of the flasks. Triplicate flasks were used for each germ for each period of exposure, and the effect of heating to 140° F. (60°C.) was tested at the end of ten, twenty and thirty minutes. The results of this heating were tested as in the preceding series, and the results are shown in table 5.

TABLE 5  
*Growth from ropy milk after heating at 140° F.*

CULTURE	10 MINUTES	20 MINUTES	30 MINUTES
B1	+	+	—
B2	+	+	—
B3	—	—	—
B4	+	+	—
B5	+	+	—
B6	+	+	—
B7	+	—	—
B8	+	—	—
B9	+	+	—
N	—*	—	—
I	—	—	—

\* Growth appeared in the material from one of the three bulbs.

The results given in table 5 show that two of the eleven cultures were completely destroyed after heating to 140° F. for ten minutes, and one of the other cultures was almost destroyed. Only six of these cultures survived a heating for twenty minutes, and all of the cultures were destroyed before the end of thirty minutes.

In considering the application of these results to commercial pasteurization, it should be remembered that the temperature was so controlled during the progress of this test as to vary not more than one half a degree from 140°. It should also be noted

that this test was conducted at the minimum temperature used in commercial pasteurization, while in commercial work during pasteurization much of the time the temperature of the milk is between 142° and 144° F. Furthermore, the development of the germ life in the milk used in this test had proceeded far beyond what would be encountered in commercial pasteurization, and the results given in tables 3 and 4 show that this more abundant growth increased the ability of the germs to withstand heating.

Accordingly, in the light of all these studies of the relation of the ropy milk germs to temperature, it seems clear that proper pasteurization of milk at 140°–145° for thirty minutes should destroy any ropy milk organisms which may be in the milk.

#### CONTROL OF THE TROUBLE

If this summary of the situation is correct, next to souring the most common change occurring in milk is the development of ropiness. Unfortunately holding milk at low temperature, which markedly checks acid development, has little restraining influence upon the development of the ropy milk organisms.

Just as sour milk is selected by many as a desirable drink, ropy milk is prized by some people as a desirable dish. However, for the city milk trade both changes are undesirable, and, therefore, it is to the advantage of the milk producer and dealer to prevent or delay both forms of change.

Where the milk is to be sold raw, the control of ropy milk is limited to preventing, as fully as possible, the ropy germs from getting into the milk. Experience has shown that the ropy organisms, when once they are introduced into a dairy, develop freely on the dairy utensils. Accordingly, when ropiness develops in connection with a given dairy, the most helpful procedure is to treat all objects coming into contact with the milk between the cow and the consumer. Where a large steam chamber is available, this treatment may take the form of live steam. Where an abundant supply of hot water is at hand, putting the utensils into hot water and bringing all parts of the utensils up

to 200° F. for a few minutes will suffice. Where both these means of treatment are lacking, recourse may be had to chemical disinfection.

A 12-ounce can of good strength bleaching powder (chlorinated lime) added to 100 gallons of water will give a powerful disinfecting solution. All pails, strainers, cloths, brushes, and other utensils which come into contact with the milk are then put into this solution and allowed to remain fifteen or twenty minutes. On removing utensils from such a solution, they should be promptly rinsed to stop the action of the chemicals.

It should be understood that this exposure to heat or to disinfectants followed by thorough washing leaves nothing on the utensils which will prevent the growth of ropy milk organisms if they find their way to the utensils after treatment. In actually carrying out such a disinfecting campaign at the farm, it occasionally happens that there is overlooked some pail, dipper, or other object which has been in contact with the ropy milk. If in connection with the handling of the milk, the germs on this one utensil spread to others, the result of the disinfection is lost and the trouble will reappear in the milk.

Where cooling tanks are used in a dairy having this trouble, the ropy germs are commonly present in the water in the tank. Accordingly, unless the tank is also disinfected, the outside of the cans will be re-seeded promptly with the ropy germs. To prevent this the cooling tank is emptied, scrubbed, and used in making the disinfecting solution described above. In such cases it is well to scrub both the tank and the interior of the milk house with the disinfecting solution.

In the actual handling of outbreaks where this work of disinfection is carefully and thoroughly done, the trouble rarely reappears. Occasionally, however, as in the case of this outbreak, there is a source of infection outside of the circle reached by this treatment, and from this source the trouble is renewed.

In the case of a company receiving milk which is to be pasteurized, the situation is somewhat different. Where the ropy milk is being furnished by one or two patrons, the treatment outlined for the dairy is usually efficient. However, when there

is an outbreak in any measure approximating the extent of the one here described, this procedure becomes expensive. It also tends in various ways to develop dissatisfaction among the patrons. Under such circumstances, it is entirely possible to handle the situation by giving attention to conditions at the milk plant.

Attention has been called to the possibility of spreading the trouble through lack of care in handling the washed cans. Every consideration in the case calls for a thorough steaming and drying of the washed cans. While increasing attention is now being given to this question at many milk plants, the present methods in most cases leave much to be desired, particularly in the drying of the cans and the can covers.

The results which have already been given show that the ropy organisms will not survive pasteurization at 140° F. for thirty minutes. Accordingly, all that is necessary in order to free the incoming milk from these germs is to be sure that the milk is all actually heated for this length of time and to this temperature. This involves careful attention to the process, particularly at the beginning and at the close of the pasteurization operations for the day.

Attention has already been called to the readiness with which the ropy germs develop upon the utensils at the dairy. They grow with equal readiness upon the utensils at the milk plant. Whenever any of them escape destruction in the pasteurizer, they may grow and make trouble. Horizontal coolers and bottlers are usually not so treated as to destroy such germs. This destruction may be best accomplished by surrounding these utensils with canvas and treating them with live steam. Milk pumps are also difficult to treat satisfactorily. The washing process should be followed by a thorough steaming of the entire milk handling system, giving particular attention to the portion between the pasteurizer and the bottle. Just before beginning operation the following morning, the entire milk line should be treated again with hot water or steam.

Attention, likewise, should be given to the milk bottles. These frequently bring the germs of ropy milk into the plant

and infect a considerable number of bottles in the washing vat. Unless the bottle washing process terminates with a treatment which brings the bottles to at least 200° F., there is a probability that these germs may develop again when the bottles are filled with milk.

Care is especially necessary in the handling of pasteurized cream held over from the preceding day which is bottled before beginning work on the milk. Where the cream is infected, the germs develop even in cold storage, and the bottling of the cream leads to a heavy infection of the bottling machinery.

When a milk plant is well seeded with the germs of ropy milk, it is quite difficult to immediately locate and destroy all of them. On the other hand, careful attention to the details of pasteurization, combined with careful steaming of all the utensils between the pasteurizer and the completion of the bottling, will at once reduce the infection to so low a point that it will cease to cause complaint among the consumers.

#### FACTORS INFLUENCING DISTRIBUTION

While ropiness, next to souring, is probably the most common change in milk, the souring of milk is almost universal. The development of acid hinders the development of the germs ordinarily producing ropiness in sweet milk with the result that raw milk held at room temperature practically always sours. As more careful attention to the handling of milk reduces the number of acid producing germs which are added to it, or the milk is held at a lower temperature which is relatively more favorable to the development of the ropy germs, or the acid producing germs are largely destroyed by pasteurization, the chances become more favorable for the development of the ropy germs.

However, ropiness in milk is the result of the growth in the milk of some representatives of a rather small group of germs. Even though the conditions in general are gradually becoming more favorable for the development of ropiness, it does not necessarily follow that the ropy germs will be at hand to take advantage of these improved opportunities. Little is known

regarding the places in nature where these ropy milk germs flourish, though there is some ground for believing that at times they may be normal inhabitants of water. However, the fact that this change in milk has been known in every land as far back as history goes suggests that the causal organisms are rather widely spread.

Recent observations have also developed the fact that ropy milk organisms are frequently present in small numbers in a milk supply which is quite acceptable to the consumers. In other words, ropy milk germs are more common members of the ordinary milk flora than is commonly understood at present, and objections are raised to them by the consumers, only when they become so abundant that their action on the milk becomes obvious.

It has been thought that the more frequent appearance of ropy milk is due to the development of new or more virulent ropy milk organisms. From the facts which have been here presented, it can be seen that this result can be even better explained on the basis of an improved handling of the milk which is directed against acid-producing germs, and which indirectly favors those producing ropiness.

In the case of the somewhat startling outbreak here recorded, its unusually wide distribution was probably due to some widespread natural source of infection, rather than to any unusual characteristic of the causal organism.

If the thesis here developed is correct, conditions for the development of outbreaks of ropy milk become more favorable as the milk is so handled as to reduce the tendency for it to become sour.

While there is little or no evidence that the causal organisms have taken on an increased vigor, this change in conditions will lead to more frequent outbreaks, unless steps are taken to counteract these new tendencies. Both on the farm and in the milk plant more care should be exercised in the handling of the utensils which come into contact with the milk. Where pasteurization is properly done, and the utensils at the milk plant are properly handled, an outbreak of ropy milk may be controlled, even where attention is restricted to these items.

The changing methods of milk production and handling are making it relatively easier for an outbreak of ropy milk to occur, but the increased knowledge of the growth conditions and temperature relations of the causal organisms are making it fairly easy to control such outbreaks.

## REFERENCES

- (1) LISTER, JOSEPH: Quart. Jour. Microscopic Sci., xiii, 380-408, 1873. (Quoted from Buchanan and Hammer.)
- (2) SCHMIDT, A.: Untersuchungen uber fadenziehende Milch. Land. Versstation xxviii, 91-109, 1883.
- (3) ADAMETZ, L.: Ueber einen Erreger der schleimigen Milch (*Bacillus lactis viscosus*). Milch-Zeitung, xviii, 941-943, 1889.
- (4) WEIGMANN, H.: Der Organismus der sogenannten langen Wei. Milch Zeitung, xviii, 982-983, 1889.
- (5) MARSHALL, C. E.: Ropiness in milk. Mich. Agr. Exp. Station Bul. 140, 1897.
- (6) WARD, A. R.: Ropiness in milk and cream. Cornell Agr. Exp. Station Bul. 165, 1899. Also Cornell Agr. Exp. Sta. Bul. 195. 1901.
- (7) COLE, L. J., AND HADLEY, P. B.: Ropy milk in Rhode Island. Rhode Island Agr. Exp. Station Bul. 136, 1909.
- (8) BUCHANAN, R. E., AND HAMMER, B. W.: Slimy and ropy milk. Iowa Agr. Exp. Research Bul. 22, 1915.
- (9) HARDING, H. A., AND PRUCHA, M. J.: An epidemic of ropy milk. Ill. Agr. Exp. Station Bul. 228, 1920.
- (10) ECKLES: Quoted from Iowa Agr. Exp. Station Research Bul. 22, p. 268.



# A COMPARISON OF THE BUTTERFAT CONTENT AND THE TOTAL SOLIDS CONTENT OF CREAMS OF VARYING RICHNESS SEPARATED FROM THE SAME SAMPLE OF MILK

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The manufacturers of ice cream for a number of years have given careful consideration to the butterfat content of cream, both from the standpoint of purchasing cream and from the standpoint of the percentage of fat in their finished product. This has been due partially to the fact that the value of the cream has been set largely by its butterfat content, and also because a minimum fat content of the finished product is set forth in legal standards. Little attention has been given to the percentage of the solids-not-fat in cream, even though the ice cream manufacturer has realized that a certain percentage was necessary to produce a good quality of the finished product. However, because of the increased cost of materials that go to make up the ice cream mix, the ice cream manufacturer has recently become keen in standardizing not only the butterfat, but the milk solids-not-fat as well.

Many analyses of milk and cream can be found in dairy literature, however very few data, if any, are available which give a comparison of the composition (fat, solids-not-fat, and total solids) of creams and the original milk from which they are separated. Various formulas have been used for estimating the amount of solids-not-fat in milk and cream, one commonly used being the pounds of milk serum (total weight of milk or cream minus the butterfat content) multiplied by 8.9 per cent. For example, using this formula there would be 6.23 per cent of solids-not-fat in 30 per cent cream.

The present investigation was carried on in order to determine the relation between creams of different fat contents obtained

from the same sample of milk; to make comparisons between the compositions of different samples of milk and cream and of creams obtained by the use of different makes of separators; and to determine whether the above stated formula is accurate enough for practical use in standardization.

Samples of milk representing four dairy breeds (Jersey, Guernsey, Ayrshire, and Holstein) and mixed milk as received at a milk plant were used, and also four different makes of separators. Normal conditions were employed in the use of the separators, i.e., the milk was separated at a temperature of 85°F., and the speed of the bowl was held at the point which the respective manufacturers recommend for maximum efficiency in separation.

The separator was adjusted to deliver a low testing cream. A sample of this cream was taken. The machine was then readjusted to deliver a higher testing cream, and a sample was taken. This procedure was repeated until four or five samples of cream—each varying in the butterfat content—were secured. These samples, along with the original sample of milk, were analyzed for butterfat by the Roesse-Gottlieb method, and for total solids by the "Official Method" (using aluminum dishes). The percentage of solids-not-fat in each sample of cream and milk was determined by the difference between the total solids and the butterfat content.

The results of 71 determinations in 14 separate runs—with the source of the supply and the separator used varying—are condensed in the following tables and graphs. The separators are designated by the letters A, B, C, and D, and not by their respective trade names.

The results show that the percentage of total solids varies in direct proportion with the increase in the percentage of butterfat (fig. 1).

The percentage of solids-not-fat decreases as the butterfat content increases, but the variation is not in any definite inverse proportion. The percentage of solids-not-fat decreases very rapidly as the butterfat content increases up to 20 per cent, but it decreases less rapidly as the butterfat increases from 20 to

Results of 71 determinations in 14 separate runs—with the source of supply and the separator used varying

LOT	SEPA- RATOR USED	SOURCE OF SAMPLE	ORIGINAL MILK			10 TO 20 PER CENT CREAM			20 TO 30 PER CENT CREAM			30 TO 40 PER CENT CREAM			40 TO 50 PER CENT CREAM			50 TO 60 PER CENT CREAM			60 TO 70 PER CENT CREAM		
			Fat	T. S.	SNF	Fat	T. S.	SNF	Fat	T. S.	SNF	Fat	T. S.	SNF	Fat	T. S.	SNF	Fat	T. S.	SNF	Fat	T. S.	SNF
1	A	Mixed				per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
			4.89	14.02	9.13							30.44	36.49	6.05	46.04	51.30	5.26	50.31	55.46	5.14			
2	B	Mixed				per cent	per cent	per cent	per cent	per cent	per cent	33.30	38.86	5.56									
			4.76	14.12	9.36							39.86	45.35	5.49									
3	A	Jersey				per cent	per cent	per cent	per cent	per cent	per cent	36.11	41.87	5.76									
		Guernsey	5.66	16.19	10.53							38.42	43.97	5.80	40.25	45.84	5.58						
4	A	Holstein				per cent	per cent	per cent	per cent	per cent	per cent	37.54	43.95	6.41	45.29	51.05	6.75						
			3.42	12.39	8.97																		
5	B	Jersey				per cent	per cent	per cent	per cent	per cent	per cent	22.63	29.04	6.40									
		Guernsey	5.81*	13.84	8.03							20.80	27.69	6.89									
6	B	Holstein				per cent	per cent	per cent	per cent	per cent	per cent	38.76	44.47	4.72	44.89	49.30	4.41	59.13	63.36	4.23			
			3.66	10.89	7.23													53.82	58.00	4.19	60.22	63.80	3.58
7	C	Holstein				per cent	per cent	per cent	per cent	per cent	per cent	28.15	33.07	4.91	38.90	43.92	5.02	43.74	48.21	4.47			
			3.71	13.17	9.46													54.28	58.28	4.01	64.69	67.59	2.90
8	B	Jersey				per cent	per cent	per cent	per cent	per cent	per cent	46.24	52.51	6.28									
			5.50	14.48	9.99													59.46	63.68	4.23			
9	A	Mixed				per cent	per cent	per cent	per cent	per cent	per cent	56.24	60.60	4.36									
			4.30	13.30	9.00													58.09	62.14	3.05			
10	B	Ayrshire				per cent	per cent	per cent	per cent	per cent	per cent	36.34	41.71	5.37	44.65	49.72	5.07	51.07	55.86	4.79			
		Holstein	3.40	12.40	9.00	13.02	20.10	7.08	25.56	33.76	8.20	39.89	43.22	3.33	46.64	51.46	4.82	51.07	56.28	5.21			
												31.15	37.10	5.96									

11	B	Holstein	3.79	13.06	9.27			25.50	31.25	5.75	38.28	43.18	4.89	41.13	46.14	5.01	50.07	54.31	4.24	
12	D	Mixed	4.20	13.89	9.68	18.41	24.76	6.35			38.24	43.64	5.41	44.77	50.02	5.26				
13	A	Ayrshire	3.45	11.60	7.15	13.63	20.70	7.07								46.53	51.98	5.45		
		Holstein				13.71	22.29	9.58												
						13.82	21.73	7.91												
						14.05	21.66	7.61												
14	B	Holstein	4.11	14.00	8.89					25.89	31.89	6.00	39.42	44.16	4.74		51.39	56.10	4.71	
										27.06	32.43	5.37								
Average.....			4.38	13.42	9.03	14.44	21.87	7.60	25.09	31.30	6.22	36.98	42.28	5.32	44.73	50.05	5.39	54.08	58.55	4.38
																				62.45
																				65.69
																				3.24

\* Specially standardized sample of milk from Lot 4.

Figure 1

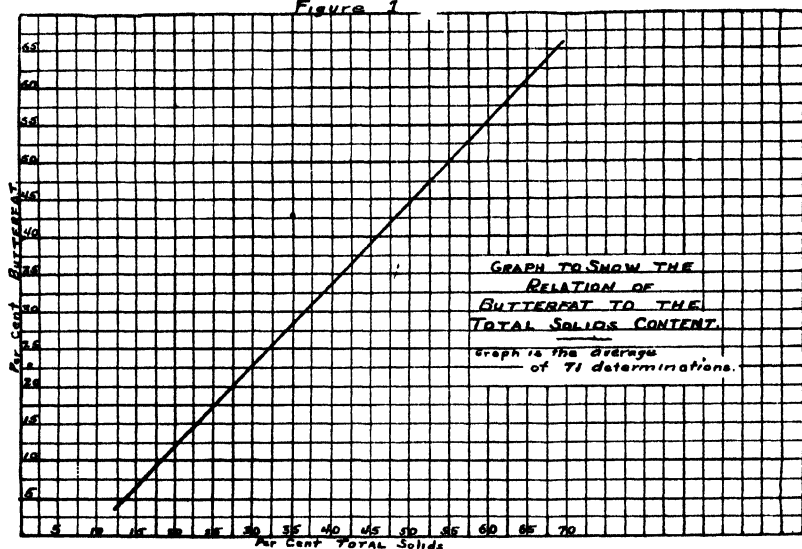
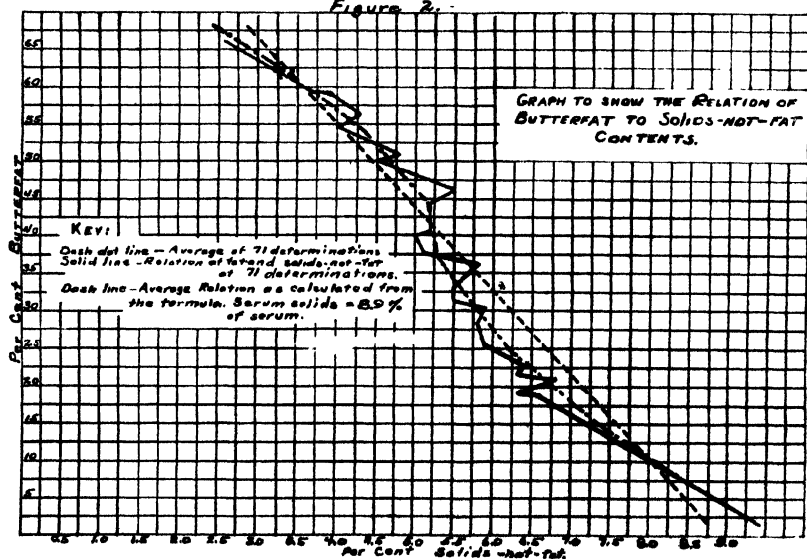


Figure 2.



45 per cent. From this point, the percentage of solids-not-fat decreases in about the same ratio with the percentage of butterfat increase, as was the case when the butterfat was increasing from 5 to 20 per cent (fig. 2).

The table shows that creams of the same butterfat content are approximately the same in their content of total solids, and of solids-not-fat regardless of the source of supply of the original milk or the separator used in the separation process. Although there is this close relationship in the case of these creams, it will be noted that the original milk of the various breeds varies with the usual marked differences. A large variation in the total solids and the solids-not-fat is shown among milks having practically the same butterfat content, but after the milks have been run through the separators, creams of practically the same butterfat content are very closely related in their content of total solids and of solids-not-fat.

The determinations show that the formula (8.9 per cent of the milk serum equals the serum solids) does not give the actual serum solids content, but can be used only to approximate the amount. The largest variation of the actual analyses from the content as calculated by the formula is 0.5 per cent. This is a large variation when one takes into consideration the fact that the solids-not-fat do not run over 6 to 7 per cent for creams which are mainly used in preparing ice cream mixes, etc., i.e., creams testing from 15 to 30 per cent butterfat. For creams of this richness, the formula figures the serum solids higher than they really are, and a material loss both financially and in the quality of the finished product may result. From the example given in the fore part of this article, a 30 per cent cream was found to contain 6.23 per cent solids-not-fat by the formula, but by chemical analyses it was found to contain 6.05 per cent, which means an error of 2.975 per cent. Thus for every hundred pounds of solids-not-fat computed by the formula, there is a loss of practically three pounds of solids-not-fat in the finished product. In the case of creams testing over 41 per cent, the chemical determinations show that the percentage of solids-not-fat (or serum solids) is larger than the amount as calculated by

the formula. For the creams testing under 41 per cent, the formula gives a higher content than is found in the cream by the analyses.

To a certain extent, the practice of considering cream as being skim milk plus the butterfat content is also erroneous. The table of analyses and the graphs show that the serum of the cream does not compare in composition to the serum of the skim milk owing to the fact that there is a variation in the rate of a decline in the amounts of water and solids-not-fat as the percentage of butterfat in the creams increases. Whether or not this is a fixed variation was not determined.

## A STUDY IN BULLS

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The high producing dairy cow of today is the result of intelligent breeding, feeding, and selection, and on these factors depends largely the future development of the dairy industry. More cows are undoubtedly needed to increase the national supply of dairy products, but, if economy of production is to receive the consideration which it deserves, better cows are of even greater importance.

It is recognized that the sire in use on a dairy farm may have a profound influence on the future usefulness of the herd and this point is being emphasized at the present time by the nationwide campaign to promote the use of better bulls. Consequently, a study of the relative values of a few bulls may not be out of place especially as it brings out some important problems in breeding.

### RÉSUMÉ OF PREVIOUS WORK

A considerable amount of work has been done to show the value of purebred bulls as demonstrated by the records of their daughters while producing under the supervision of the Breed Associations and the Experiment Stations. This work is valuable but it need not be considered here as it deals with selected populations—cows which did not come up to certain standards, set by the Breed Associations, being automatically eliminated. Work with unselected populations is more limited.

In his work with the Jersey herd at the University of Missouri Eckles (1) has shown the differences which occur in the abilities of bulls to sire daughters capable of good production.

In the Missouri work no age allowance was made but where only a limited number of records for the heifers were available they were compared with the corresponding records of their



dams. There are vast differences in the values of these bulls so far as the producing abilities of their daughters are concerned. The daughters of the best bull produced 61 per cent more butterfat than did their dams while those of the least valuable bull gave 26 per cent less fat than their dams. Some of the bulls had daughters which differed very little from their dams in producing ability.

TABLE 1

*A comparison of the Jersey sires used in the University of Missouri herd*

SIRE	DAMS				DAUGHTERS				INCREASE IN PRODUCTION	
	Number of cows	Number of lactations	Average production		Number of cows	Number of lactations	Average production		Milk	Fat
			Milk	Fat			Milk	Fat		
			lbs.	lbs.			lbs.	lbs.	per cent	per cent
Missouri Rioter .....	4	23	5380	234	4	26	4381	216	-19	-8
Hugorotus.....	11	62	4969	231	11	50	4576	245	-8	6
Lorne of Meridale.....	12	66	4559	231	12	67	5969	287	31	30
Missouri Rioter 3rd..	3	14	4775	238	3	15	8005	384	68	61
Minette's Pedro.....	20	66	5321	268	20	66	5376	271	1	1
Brown Bessie's Registrar.....	5	8	6029	293	5	8	4295	217	-29	-26

It is reported from the Ohio Station (5) that one Holstein bull in the station herd had daughters which produced 1299 pounds more milk and 40 pounds more fat than their dams while a Jersey bull in the same herd had daughters yielding 700 pounds less milk and 45 pounds less butterfat than their dams.

Work reported by Kildee and McCandlish (2) showed that purebred sires did much in one generation to develop a herd of good producing cows from a foundation of scrubs and later results on this project by McCandlish, Gillette and Kildee (4) showed that the use of purebred sires on a scrub herd could double the milk and butterfat production in two generations.

Further interesting information was obtained in this work regarding the variations in the ability of two Guernsey bulls to sire good producing daughters. The Missouri work shows that some purebred sires are not good enough to head a purebred

herd and the Iowa results clearly demonstrate that some pure-bred sires should not even be used on scrub or grade cows. The daughters of one of the Guernsey bulls under consideration produced 35 per cent more butterfat than did their scrub dams while the daughters of the other bull produced only 2 per cent more fat than their scrub dams.

Lawritson and his co-workers (3) discussed the value of a Jersey and two Holstein bulls used at the Nebraska Station.

TABLE 2  
*A comparison of sires used at the Nebraska Station*

SIRE	DAMS				DAUGHTERS				INCREASE IN PRODUCTION	
	Number of cows	Number of lactations	Average production		Number of cows	Number of lactations	Average production		Milk per cent	Fat per cent
			Milk	Fat			Milk	Fat		
			pounds	pounds			pounds	pounds		
<i>Jersey</i>										
Golden Shylock.....	9	21	6,491.1	345.7	12	21	8,496.9	466.1	31	35
<i>Holstein</i>										
Prince Ormsby Mercedes DeKol.....	3	6	13,704.5	435.6	3	6	17,677.2	660.6	29	52
King Segis Hengerveld Vale.....	5	8	13,630.7	454.2	5	8	17,261.5	600.0	27	32

The first and second lactation periods of the daughters of these bulls were compared with the corresponding periods of their dams. For the purposes of comparison and uniformity an age allowance has been made for those records and where immature records for the dams are not available the records of the daughters have also been deleted. The records used here are therefore on a different basis than that on which they were originally reported.

In this work it was found that each of the three bulls discussed had a considerable influence in increasing the productivity of the herd.

## SOURCE OF DATA

This study comprises a summary of the results obtained through the use of bulls in the purebred herds on the Iowa State College Dairy Farm since its establishment as a separate unit in 1907. Some bulls, including a few that were used for a short time only, have had but one daughter complete one or more lactation periods on the farm, and so the data concerning these have been excluded as valueless.

All normal records completed by the purebred daughters of the other bulls have been included and the records of these cows are compared with the records of their dams. Where records are of long duration only the first 360 days are considered.

TABLE 3  
*Percentage of mature production expected of immature heifers*

AGE	PERCENTAGE OF MATURE PRODUCTION
<i>years</i>	
1	70
2	80
3	85
4	95

To make the records comparable an age allowance has been introduced. All records are computed to the mature—five-year old—basis and the scale of allowances for different ages is based on a study made at the Iowa Station of over 10,000 yearly records.

Unfortunately the numbers of daughters of the various bulls are in some cases not large, but some indication of the value of the bulls can be obtained from the records.

## DISCUSSION OF RESULTS

The five bulls of four dairy breeds that were used in this work were evidently of different values so far as their abilities to sire good producing daughters were concerned. It is not possible to rank the bulls absolutely according to their abilities as can easily be seen from a study of the records of the two generations

**TABLE 4**  
*Summary of results with sires used at Iowa State College*

SIRE	DAMS				DAUGHTERS						TOTAL INCREASE IN PRODUCTION			PERCENTAGE INCREASE IN PRODUCTION		
	N cows	N lactations	Average production			N cows	N lactations	Average production			Milk	Fat	Fat	Milk	Fat	Fat
			Milk	Fat	Fat			Milk	Fat	Fat						
pounds	pounds	per cent	pounds	pounds	per cent	pounds	pounds	per cent	lbs.	lbs.	per cent					
<i>Ayrshire</i>																
Willowmoor Robin Hood 19th.....	3	13	8,932.3	330.44	3.70	3	4	9,666.4	432.03	4.47	734.1	101.59	0.77	8	31	21
<i>Guernsey</i>																
Imp. Rouge II's Son... <i>Holstein</i>	5	46	6,630.9	286.98	4.33	8	14	7,253.5	357.42	4.93	622.6	70.44	0.60	9	25	14
Spring Farm King Pontiac 8th.....	6	23	13,051.9	457.50	3.51	6	12	13,433.9	470.43	3.50	382.0	12.93	-0.01	3	3	0
<i>Jersey</i>																
Fox's Lad O'Dream-wold .....	3	13	7,667.9	356.30	4.65	3	7	6,005.0	290.84	4.84	-1,662.9	-65.46	0.19	-22	-18	4
Pogis 80th of Hood Farm.....	7	46	7,280.4	343.25	4.71	9	19	7,504.6	393.65	5.26	224.2	50.40	0.55	3	15	12

of females considered and no breed distinctions should be attempted. This is further emphasized by the fact that within one breed are two bulls, one of which produced a 15 per cent increase and the other an 18 per cent decrease in the fat production of the herd.

Three of the bulls considered were valuable as herd improvers as their daughters produced from 15 per cent to 31 per cent more butterfat than did their dams: The daughters of one produced only 3 per cent more milk and fat than did their dams so he did not work any marked improvement, but it should be remembered that the cows to which he was mated produced on the average over 100 pounds more butterfat per year than did the cows to which the other bulls were mated. The remaining bull was a detriment to the herd as his daughters produced 22 per cent less milk and 18 per cent less fat than did their dams.

It is very generally considered that milk production can be changed more rapidly than can the yield or percentage of butterfat but in the cases under consideration the reverse proved to be true as a rule.

#### THE INDIVIDUAL BULLS

The individual bulls do not need consideration in great detail as a general rule but it may be said that the three daughters of the Ayrshire bull, Willowmoor Robin Hood 19th, were all out of different dams and two of them exceeded their dams in producing ability while the other was of about the same capacity as her dam.

The Guernsey bull, Imp. Rouge II's Son, had eight daughters out of five different cows and on the average they produced 25 per cent more butterfat than their dams. They varied greatly in producing ability, however, the average production of the best one being 400.58 pounds, while that for the poorest was 271.79 pounds of fat. There seem to be two main reasons for this and these will be discussed later.

The daughters of the Holstein bull, Spring Farm King Pontiac 8th, were all from different cows and in five cases they were better producers than their dams.

The three daughters of the Jersey bull, Fox's Lad o'Dreamwald, were all from different cows and in each case they were poorer producers than their dams. The other Jersey bull, Pogis 80th of Hood Farm had nine daughters out of 7 cows and in all but three cases they were better producers than their dams.

#### INDIVIDUALITY AS A PRODUCTION DETERMINANT

The individual cow must be looked on as the unit when milk production is being considered and its great importance is emphasized by a few cases found in the present study.

From a survey of the records given in table 5 it can be seen that animals which were full sisters varied greatly in their producing ability on several occasions. The two cows 247 and 298, out of cow 97 and by Imp. Rouge II's Son were both much better producers than their dam and were quite uniform in production. When two other daughters of Imp. Rouge II's Son are considered, namely cows 225 and 267 out of cow 98, a quite different set of conditions are found as cow 225 produced the same amount of fat and 14 per cent less milk than her dam while cow 267 produced 35 per cent more milk and 61 per cent more butterfat than her dam.

A similar condition is found in the case of some of the daughters of the Jersey bull Pogis 80th of Hood Farm. The two cows 201 and 289 were full sisters, out of cow 13 and while cow 289 produced 26 per cent more fat than her dam cow 201 produced only 6 per cent more. Another pair of full sisters were cows 194 and 223 out of cow 127 and while cow 194 produced 54 per cent less fat than her dam, cow 223 produced 10 per cent more.

These facts clearly show that the factor of individuality is of exceedingly great importance in determining the production of an animal—in fact it is the greatest factor, others being only subsidiary. Two animals may be of the same "breeding" but of absolutely different powers so far as their "individual" producing ability is concerned. It must be admitted however that the greater the attention given to the selection of breeding stock for a number of generations the less will be the variation in producing ability of the individual animals of the same or similar breeding.

TABLE 5  
The influence of individuality on production

SIRE	DAMS				DAUGHTERS				PERCENTAGE INCREASE IN PRODUCTION		
	Cow number	Average production			Cow number	Average production			Milk	Fat	Fat
		Milk	Fat	Fat		Milk	Fat	Fat			
									pounds	pounds	per cent
Guernsey	97	5288.9	229.42	4.33	247	8385.4	400.58	4.78	57	75	10
					286	8309.3	394.74	4.75	57	73	10
					322	8908.5	470.70	5.28	68	105	22
Imp. Rouge II's Son	98	7258.7	294.87	4.06	225	6213.0	294.16	4.73	-14	0	16
					267	9772.8	473.38	4.84	35	61	19
Jersey	13	8245.0	380.46	4.62	201	8735.2	401.55	4.60	6	6	0
					289	8345.5	479.30	5.74	1	26	24
Pogis 80th of Hood Farm	127	8190.5	382.92	4.68	194	3345.3	175.68	5.25	-59	-54	12
					223	7837.6	419.89	5.36	-4	10	15

## "NICKING"

When animals are mated and the offspring resulting approaches the ideal which is being sought the animals mated are said to "nick" well. The problem of nicking is not given due consideration and in many cases a bull is discarded as useless because he did not give satisfactory results when mated to a certain group of cows, whereas, if he had been mated to other animals the results might have been excellent on account of the proper "nicking" taking place. This is well demonstrated in the case of the daughters of the Guernsey bull, Imp. Rouge II's Son.

At present records are available on 8 daughters of Imp. Rouge II's Son out of 5 cows and some good illustrations of the value of nicking can be found by dividing the daughters of this bull into two groups—those from cows producing less than 300 pounds of butterfat per year and those from cows producing more than this amount.

The two cows, 97 and 98, producing less than 300 pounds of butterfat per year had an average production of 264.27 pounds, while their 5 daughters—except No. 225 which just equalled her dam in fat production—exceeded their production by a considerable margin, the average increase being 47 per cent.

The three cows, 123, 186, and 187 produced on the average over 300 pounds of butterfat yet the daughters of these cows were in every case poorer than their dams, the average decrease being 10 per cent in butterfat yield.

If Imp. Rouge II's Son had been mated only to the cows in group II he would have been classed as of no value but his daughters out of the cows in group I were not only better producers than their dams but they were also better than the cows of group II. Consequently, it must be deduced that so far as milk and butterfat production were concerned Imp. Rouge II's Son did not nick well with the cows in group I. This fact must never be neglected in judging the merits of a bull for breeding purposes.



TABLE 6  
 "Nicking" as illustrated by the daughters of Imp. Rouge II's Son

GROUP	DAMS				DAUGHTERS				PERCENTAGE INCREASE IN PRODUCTION		
	Cow number	Average production			Cow number	Average production			Milk	Fat	Fat
		Milk	Fat	Fat		Milk	Fat	Fat			
		pounds	pounds	per cent		pounds	pounds	per cent	pounds	pounds	per cent
I	97	5288.9	229.42	4.33	247	8385.4	400.58	4.78	57	75	10
					286	8309.3	394.74	4.75	57	73	9
					322	8908.5	470.70	5.28	68	105	22
	98	7258.7	294.87	4.06	225	6213.0	294.16	4.73	-14	0	17
II	123	6326.9	322.22	5.09	267	9772.8	473.38	4.84	35	61	19
		186	6854.9	312.41	292	5546.8	271.79	4.90	-12	-16	-4
		187	8328.7	365.19	254	5264.5	282.71	5.37	-23	-10	18
					226	6650.3	335.46	5.04	-20	-8	15
Average group I		6305.5	264.27	4.19		8019.2	398.42	4.84	27	47	16
Average group II		7303.6	336.14	4.60		5875.3	301.62	5.14	-20	-10	12
Grand average		6331.0	286.98	4.53		7253.5	337.43	4.93	9	25	9

## SUMMARY

1. Purebred dairy bulls vary greatly in their ability to transmit producing capacity to their offspring.
2. This is not a breed characteristic.
3. In determining the value of a bull the difficulties he has to overcome must be taken into consideration. When a bull is mated to high producing cows it is not always easy to have the next generation producing much better than their dams.
4. Animals of the same or similar breeding may vary greatly in producing ability due to individual characteristics.
5. Difficulty will sometimes be found in getting a bull that will "nick" properly with all the cows in a herd.
6. If the production of a herd is to be increased rigid selection on the basis of production must accompany intelligent breeding.

## REFERENCES

- (1) ECKLES, C. H. 1911 Dairy cattle and milk production.
- (2) KILDEE, H. H., AND McCANDLISH, A. C. 1916 Influence of environment and breeding in increasing dairy production. Bull. Ia. Agr. Expt. Sta. 165.
- (3) LAWRIE, M. N., HENDRICKSON, J. W., AND NEVENS, W. B. 1919 Purebred Sires Effect Herd Improvement. Cir. Neb. Agr. Expt. Sta. 8.
- (4) McCANDLISH, A. C., GILLETTEE, L. S., AND KILDEE, H. H. 1919 Influence of environment and breeding in increasing dairy production. II. Bull. Ia. Agr. Expt. Sta. 188.
- (5) Building up the dairy herds of Ohio. 1913 Cir. Oh. Agr. Expt. Sta. 135.

## BACTERIAL CONTROL IN MILK PLANTS

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Milk inspectors and health officials should not rest assured of a safe product because of the mere presence of a milk pasteurizing plant in their city. Special attention must be given to the operation of such a plant in view of the fact that unless it is properly operated it may become a chance source of infection to the community.

In many instances, only an attempt at the process of pasteurization is being made. This is due to either a lack of proper attention and responsibility or to the absence of understanding, or the part of the operators, as to the functions and reasons for the process.

Bacteriological control of the process of pasteurization and of the condition of the subsequent containers and contact surfaces is essential. This examination should not be left entirely to the pasteurizing plant, but should be made frequently by competent officials under the direction of milk inspectors or health officials who are responsible for the condition of the milk supply and of the health of the community.

In considering the efficiency of various pasteurizing processes, it should be understood that the percentage reduction of bacteria in raw milk by heating does not form a measure of safeness of the final product. After heating, the so-called pasteurized milk is changed from its original condition and any inoculation subsequent to the heating introduces an uncertain element which may be of concern and which may void the reduction in bacteria already secured by heating the milk.

In the examination of milk to determine the percentage of reduction due to the heating process, a series of samples should be taken aseptically at intervals at the points representing definite stages in the pasteurizing process. It is only in this manner

that undue changes in the bacterial content can be noted and corrections made.

An accurate thermometer should be used to note the temperature at the various stages.

The first series of samples should be secured from raw milk entering the heating apparatus.

After the heating and holding process, in which temperature and time should be noted, another series of samples should be secured in the same manner at the point where the milk leaves the holder. This point of sampling varies with different apparatus and especially when the enclosed type of holder is used so that it is not always possible to secure samples immediately after the milk is held.

If milk is pumped, a series of samples should be secured before and after the pumping process.

Following the holding process, milk is usually run over a cooler and as it comes from the cooler, a series of samples should be secured and temperature noted.

The cooling process is usually followed by the bottling and a series of samples from the bottled milk should be secured and temperature noted.

In some plants the bottled milk is stored for a certain period in a refrigerator room prior to delivery. Sometimes this is an over-night storage and in such instances a series of samples of the held-over milk should be secured and the time of storage and temperature of the milk and the temperature of the storage room noted.

In general then we have the following points where a series of samples should be taken at intervals:

1. Raw milk at entrance to heater. (Temperature to be noted.)
2. Heated milk after being held. (Time and temperature to be noted.)
3. Milk as it comes from cooler. (Temperature to be noted.)
4. Milk in bottles or cans. (Temperature and time of delay before placed in storage to be noted.)

5. Milk in bottles or cans after storage. (Temperature of milk and air in storage room, and length of storage period to be noted.)

All samples should be iced and removed to the laboratory for dilution and plating as soon as possible. A chart showing the different stages where samples were secured can be made and the percentage reduction at each stage can be ascertained when the bacteria counts are known. A series of preferably five samples taken at intervals of several minutes at each point is essential in order that the true condition of the milk may be known throughout the process.

Using the average of the raw milk counts as a basis, the reduction at the various points can then be computed. A record of milk temperature, time of holding and of delays in handling, time of storage with temperature of storage room, are necessary for complete information.

It is also essential that a check be made on any automatic time and temperature controlling device that may be present. By immersing the recording thermometer bulb in the same liquid with an accurate tested hand thermometer for a period of time, a comparison can be secured. Any automatic time-holding device should be checked by comparing the time of holding with an accurate watch. Continuous holding apparatus can be checked by noting the time required to fill or to empty the holder. The length of holding sometimes depends on the capacity and speed of a milk pump, the speed being under control of a steam valve. By proper regulation of the speed of the milk pump, the time of holding may be controlled to some extent.

In the case of vat holders, the length of time the entire batch of milk is held at the pasteurizing temperature should be checked. When time is required for the vat to become filled and emptied, only the length of time the entire quantity of milk is held at the pasteurizing temperature should be considered. Recording thermometer charts usually record from the time the milk enters the vat and in the interpretation of the chart record, the time when the vat becomes filled should be known and the time

of holding the milk at the proper temperature gauged from that point to the time the vat starts to be emptied. This may result in some milk being held longer than the required time because of the time of emptying the vat, but it is the only positive way to insure the holding of all the milk at the proper temperature for the desired period of time.

#### INTERPRETATION OF RESULTS

The following table has been taken as an example for interpreting results from similar tests at many pasteurizing plants. The tests were made over a period of five days and are the averages of 128 samples.

Raw milk after clarifying:		
Temperature.....	84°	
Count.....	152,375	
Heated and held:		
Time.....	30 minutes	
Temperature.....	145°	
Count.....	6,240	
Reduction.....	96 per cent	
Off cooler:		
Temperature.....	45.2°	
Count.....	8,452	
Reduction.....	94.4 per cent	
Bottled:		
Temperature.....	51°	
Count.....	15,592	
Reduction.....	89.7 per cent	
Stored twenty-four hours:		
Temperature.....	42.2°	
Count.....	24,386	
Reduction.....	83 per cent	

	TEMPERATURE	BACTERIA COUNT	PER CENT BACTERIA COUNT INCREASE
Increase due to milk passing over cooler...		2212	35.4
Increase due to milk passing through bottler.....	5.8°	7140	83.4
Increase due to milk being stored twenty-four hours (*temperature decrease).....	*8.8°	8894	57.0

From the time of maximum reduction (96 per cent) to time the milk was in the bottle, reduction—89.7 per cent—there was a difference of 6.3 per cent in reduction. This represents a total increase in temperature of 5.8°, and a bacteria count increase of 9352 per cubic centimeter or 149.8 per cent.

From the time of maximum reduction (96 per cent) to the time of delivery, after twenty-four hours storage, reduction—83 per cent—there was a difference of 13 per cent in reduction. This represents a total decrease in temperature of 3° and a total increase in bacteria of 18,146 per cubic centimeter or 290.8 per cent.

The reason for the increases should be sought, as there is evidence of a waste of energy in allowing increases to occur after a certain degree of reduction has been gained.

Since the milk, after being reduced in bacterial content (96 per cent), has passed over the surface of a cooler, that is the first point where attention to cleanliness and sterilization should be centered. Exposed surfaces always allow access for dust particles and bacteria which may be adhering to the same. Improper washing and sterilization of pipes and cooler surfaces allow milk to collect and sour and dry in crevices and joints. Milk passing through or over such accumulations become inoculated. This is easily noted when the first milk over the cooler shows an excessive increase in bacterial content. A bacterial examination of sterile water run over the cooler prior to the milk-cooling operation will reveal the source of trouble.

#### PUMPS

If milk is pumped, the condition of the inside of the pump may cause inoculation of milk. Securing the bacterial condition of a series of milk samples secured before and after the pumping process, will determine the increase due to the agitation or inoculation caused by the pump. A bacterial examination of sterile water run through the pump when it is not in use may reveal a deposit of sour or dried milk which has become an inoculating agent.

## BOTTLING MACHINE

The bottling machine in a milk plant is sometimes a difficult piece of equipment to clean properly. The rubber parts and filling mechanism should be taken apart and thoroughly cleaned after each day's run. If valves are not removed, an accumulation of drain-water occurs, and unless this is disposed of before the filling begins, the first bottles filled may show excessive bacterial counts as well as added water. Such a condition has been noted quite frequently and is due to carelessness. An instance has been noted of the use of a strong disinfectant, in an attempt to sterilize the bottling machine and the presence of the disinfectant in the first milk bottled and delivered to consumers. This shows the extent of carelessness that may be attained in this respect.

A bacterial examination of the drain-water or of sterile water run through the filling valves will help to determine the source of inoculation.

## BOTTLES

Custom rules that the transparent glass bottle is the most common container for milk. Custom seems to also rule that it is the most misused container among the perishable food containers which are used for marketing purposes. Economy rules that the cost of a glass bottle requires it to be used over and over in order that its value may be realized.

It is a discouraging thing to know that sometimes when an empty milk bottle looks to be clean, in reality it is not. It is this deception which causes many bottles to be refilled with milk with no special attention being given to their washing and sterilization.

The washing and sterilization of returned empty milk bottles in a city milk plant has become a difficult and expensive problem. In order to secure the best results, there must be several inspections and then a final inspection for the detection of the visibly unclean bottles so that the business may not be injured by loss of trade or publicity because of unclean containers.



Returned bottles should be sorted before any attempt at washing is made. Those which contain visible dirt should go to the soaking machine for special attention, and those which are passed as washable should go to the rinsing, washing and steaming machines, which have quite recently been developed to a high degree of proficiency. The makers of bottle-washing machines do not claim that unwashable bottles can be made clean, neither do they insure the condition of the bottles after they leave the washing machine. Improper storage or handling may void the utmost previous precautions against inoculation.

Bacterial tests for the condition of the empty bottles which are to be filled, should be made frequently. The method employed usually consists of rinsing empty bottles with about 30 cc. of sterile water. Test tubes containing this quantity can be prepared in the laboratory and taken to the milk plant. Bottles ready to be filled are selected at random from the supply and the 30 cc. of sterile water poured in each. A milk bottle cap is then placed in each bottle and a thorough shaking given. Some of the rinse water from each is then drawn off into sterile test tubes and a bacteriological examination made of it in the laboratory. The total count of 1 cc. of the rinse water can be determined and then the bacterial condition of the bottle as affecting 1 cc. of milk poured therein, can be computed. While the inoculation per cubic centimeter of milk may in some instances be small, it must be remembered that the results show inoculation which may develop under favorable conditions with a detrimental effect on the milk.

The inoculation of the milk from the milk bottle itself can be reduced to a minimum by thorough washing, steaming and draining prior to filling. Such reduction of the chances of inoculation should be the object of milk plant operators and milk inspectors alike.

Under various conditions of washing and steaming of milk bottles at different milk plants, the inoculation per 1 cc. of milk that would come from bottles varied as the conditions varied. In order to show the effect of different methods, the following tables were prepared by differentiating between con-

ditions where good methods were in practice, and conditions other than good which were associated with high inoculation.

*Bottle washing (no mechanical washer or steamer)*

PLANTS	BOTTLES	AVERAGE INITIAL INOCULATION PER CUBIC CENTIMETER
9	30	2636.0
1	15	1192.4
1	20	568.0
1	10	395.0
7	90	167.0
1	25	78.1
20	190	Average 458.2

Range of initial bacterial inoculation, 0 to 9005.

*Mechanical washer and steamer present*

1	12	238.0
1	8	149.0
1	10	137.0
1	10	115.0
3	24	92.9
2	45	72.3
1	10	4.8
1	20	Less than 1
1	15	Less than 1
12	154	Average 78.8

Range of initial bacterial inoculation, less than 1 to 900.

Total plants..... 32  
 Total bottles..... 344  
 Average initial inoculations, all conditions..... 288.3  
 Difference of 82.8 per cent in favor of mechanical washer and steamer.

The illustration shows that when mechanical bottle washers and steamers were used and special attention paid to the inspection and storage after washing, that the average inoculation per cubic centimeter was 78.8, the range being from less than 1 to 900. When other than these conditions for washing were used, the results showed a higher average and a wider range.

Taking all conditions, good and poor, of washing and steaming bottles at city milk plants, as shown by the following table,

the average inoculation per cubic centimeter secured by examining 344 bottles at 32 plants, was 288.3, varying from less than 1 to 9005.

Under commercial conditions, it seems reasonable to expect the average bacterial inoculation of bottles to be confined within the average range secured when standard mechanical washers and steamers are in use, and care is given to storage prior to filling. Should the bacterial inoculation fall outside this average, it would be well to consider changes in methods and equipment, and to devote more attention to this part of the plant operation.

An experiment was conducted at one milk plant to determine to what extent if any, the bacterial condition of bottles would be changed by rinsing with cool water just prior to filling. In this instance, the rinsing was desired because of a faulty storage consisting of upright bottles and a warm room. The following table shows the results of the tests on a case of bottles half of which were tested before rinsing and the other half being tested after rinsing.

BEFORE RINSING		AFTER RINSING	
Total bottle count	Average initial inoculation per cubic centimeter milk	Total count	Average initial inoculation per cubic centimeter milk
120,000	240.0	16,000	3.2
3,840	7.5	5,200	6.4
15,200	31.0	2,000	4.0
31,200	62.4	4,800	9.6
44,400	88.0	6,600	13.6
42,928	85.8	3,720	7.4

Difference due to rinsing, 91.4 per cent.

While a reduction of 91.4 per cent was secured by the rinsing and a slight cooling of the bottle resulted, the operation may be called impractical in that it constitutes an added expense of water and of equipment and handling, as a substitute for correct storage by inverting bottles in a cool room. It also causes the handling of wet cases and bottles at the filling machine and

because of the rinsing, admits a chance for inoculation if the rinse water becomes contaminated.

#### CANS

The initial inoculation per cubic centimeter of milk which may be given when it is poured into a milk can should be a matter of concern. The inoculation thus acquired is governed by the condition and number of cans used in the transportation and sale of the product, and in many instances the degree of inoculation becomes sufficient to seriously injure or ruin the milk.

Since the milk can is the recognized last container which is used for transporting milk between the milk shipper and the city dairy, the responsibility of the condition of this container should be definitely fixed. Inoculation from cans varies from the efficiency of washing, steaming and drying and the length of time between the washing process and filling of cans with milk.

The proper apparatus to be used in efficient can washing seems to be a wash tank with rushing facilities, rinsing tank, steam jet and air blast. Accompanying this equipment, there must be a proper storage space. No system of handling the cans is complete unless the metal can covers and lids or wooden plugs receive a thorough washing and drying.

In the examination of milk cans under various washing and steaming conditions, it has been found that while some of the essential equipment was present, the entire operation was carried on so hurriedly as to make the method ineffective.

The usual procedure in the bacterial examination of empty milk cans is to secure a sample of drain water in the can, if sufficient is present, otherwise 200 cc. of sterile water is poured into the can. After a thorough rinsing, a sample of the rinse water is secured by pipette, placed in a sterile test tube and removed to the laboratory for bacterial examination. If the drain water is sampled, the count per cubic centimeter is multiplied by the quantity of the drain water to secure the total number of bacteria in the can. If the sterile rinse water (200 cc.), has been introduced into the can, the bacteria count of the sample should

be multiplied by 200 in order to secure the total number of bacteria in the can.

When the total bacteria per can is known, the number is divided by the volume of the can in cubic centimeters, in order to determine the probable initial inoculation per cubic centimeter that would result if the can were to be filled with milk or other liquid.

The following tests were carried on under three conditions classified as follows:

1. Cans delivered to railroad for shipment to producers from plants having wash tanks, washing powder, hand brushes, and steam jets for washing and steaming. The cans were both wet and dry inside.

2. Freshly washed cans at city milk plants having tanks, washing powder, hand brushes, and steam jets. All cans slightly moist.

3. Freshly washed cans at milk plants having mechanical washing machine and air blast for drying cans.

### *Condition 1*

Cans from milk plants where tanks, hand brushes, cleansing powder, and steam jets were present. Examined at the railway station as they were being sent to shippers.

PLANTS	TOTAL CANS	CONDITION	AVERAGE INOCULATION	RANGE
29	146	Wet: 108 Dry: 38	547,994 1,870	52 to 4,332,000 2 to 19,632

Inoculation in wet cans 99.7 per cent more than in dry cans.

In conjunction with the above conditions, it may be interesting to note the effect of a complete change of method and equipment such as was installed in one milk plant during the course of the investigations.

Bacteria counts were made on a series of 10 cans as they were delivered to the railroad, before the new apparatus was installed, as follows:

CANS	AVERAGE AMOUNT OF DRAIN IN CANS	AVERAGE INOCULA- TION PER CUBIC CENTIMETER	RANGE OF INOCULATION
	cc.		
9	83	88,500	23,700 to 178,000

After the can-washing machine with attached air blast was installed, bacteria counts were made on a series of 12 cans delivered to the railroad as follows:

CANS	CONDITION	AVERAGE INOCULATION PER CUBIC CENTIMETER	RANGE OF INOCULATION
12	5 dry 7 damp	122	52 to 634

Difference of 99.8 per cent in condition of cans delivered to the railroad for shipment, due to the installation of can-washer, steamer and dryer.

### *Condition 2*

Freshly washed cans at city milk plants, just prior to filling or shipping back to producers. Tanks, hand brushes, cleansing powder and steam jets were present. All cans slightly moist inside.

PLANTS	TOTAL CANS	AVERAGE INITIAL INOCULATION
4	29	684.0
10	131	86.7
3	6	1564.0
1	11	196.7
6	30	162.0
1	16	20.7
1	11	205.8
1	10	18.7
27	244	Average 614.0

Range of initial bacterial inoculation, 5.2 to 2750.

In conjunction with the above conditions, it became possible to secure comparative tests when one milk plant installed a can steamer during the investigation.

Bacteria tests were conducted on a series of 10 freshly washed cans when no facilities for thorough steaming were present:

CANS	CONDITION	AVERAGE INITIAL INOCULATION PER CUBIC CENTIMETER	RANGE
10	Slightly moist	206.3	60 to 675

After a new can steamer was installed, a series of bacterial tests was made on 9 freshly washed cans as follows:

CANS	CONDITION	AVERAGE INITIAL INOCULATION PER CUBIC CENTIMETER	RANGE
9	Slightly moist	97.3	1.5 to 441

The series of tests showed a difference of 53 per cent in favor of the installation and use of a can steamer.

### *Condition 3*

Freshly washed cans at milk plant. Washing machine and air blast used for washing and drying cans.

PLANTS	CANS	AVERAGE INITIAL INOCULA- TION PER CUBIC CENTIMETER	RANGE OF INOCULATION
2	10	18.6	0 to 62
1	34	9.7	0 to 331
1	16	20.2	1.3 to 91.5
4	60	Average 14.0	Range 0 to 331.0

From the foregoing conditions, it may be concluded that it is possible and thoroughly practicable to secure a comparatively low initial inoculation in milk cans when the proper apparatus is installed and in constant daily use.

### MILK BOTTLE CAPS

The milk bottle cap may well be considered as a part of the final container of milk. While it may not be possible to measure the degree of inoculation given to milk by the bottle cap, it must not be overlooked as a source of inoculation.

The possibility of inoculating milk by introducing infectious disease germs from the fingers of persons inserting milk caps into bottles, must be recognized as a chance source of infection.

The use of machine cappers for inserting caps and the proper storage of the caps in sealed tubes, must be recommended as much more preferable than capping by hand. The bacterial condition of milk-bottle caps poorly stored in bulk lots and exposed to moisture, dust and flies has been compared with bottle caps properly stored in sealed tubes ready for the capping machine. While the initial inoculation is necessarily small because of the limited surface in contact with milk, nevertheless, a difference of 88.8 per cent in initial inoculation per cubic centimeter of milk in favor of caps in sealed tubes, was noted.

The usual method of procedure in determining the bacterial condition of milk bottle caps is as follows:

Secure a bunch of from 20 to 30 bottle caps as constituting a fair sample of those being used, taking care not to inoculate the surfaces. In the laboratory, the entire number of caps are placed in a sterile glass beaker.

Sterile water, 100 or 200 cc., is then poured over the caps and a sterile pipette or rod, used to separate and stir the contents for several minutes. A sample of the wash water is then drawn into a sterile pipette and an examination carried on the same as for bottle and can rinse water.

The total bacterial count per cubic centimeter is multiplied by the quantity of sterile water used, and the total count secured is then divided by the number of caps tested. This will give the approximate number of bacteria per cap, but as only one-half of the cap surface is exposed to the milk in the bottle, it is necessary to divide again by 2, to secure the approximate initial inoculation, resulting from the cap contact surface.

It is not uncommon to find the initial inoculation per cubic centimeter of milk ranging from 0 to 400 for each cap, but the usual limits, where care is taken in storage, may be found between 0 and 6.



## CONCLUSION

Bacterial control of pasteurization and of the factors which may subsequently affect the milk, are essential to the industry. Laboratory facilities where bacterial tests may be conducted should be associated with every milk plant, because of the check that is thereby made possible on equipment and labor and on the quality of the product received and the product delivered.

The initial inoculation given to milk, resulting from the bacterial condition of exposed surfaces, coolers, bottling machines, pumps, pipes, milk bottles, milk cans, and bottle caps should be reduced to the minimum. This is only possible when strict daily attention is given to cleaning and sterilizing these contact surfaces.

The knowledge of the degree of development of bacteria under favorable conditions causes a realization that initial inoculation given to milk may ultimately spoil it, and from an economical standpoint, at least, precaution is of value.

Under proper conditions, it is possible and thoroughly practical on a commercial scale to secure in subsequent containers, a comparatively small range of initial inoculation. This is evidenced from the foregoing tests which are fairly indicative of common practices, as found during investigations, observations and bacterial examinations, personally made, in 92 milk plants located in 27 cities.

After a certain degree of reduction in bacteria content of milk has been secured by heating and holding for the specified time, it is poor business to allow the result to be made void or nearly so because of subsequent inoculation. \*

The process of pasteurization of milk and all of the factors which may influence the milk after it has been pasteurized should be under bacterial control, either by the milk-plant bacteriologist or an experienced bacteriologist employed by the city board of health, for it is only when control is present that desired results are obtained.

# ABSTRACTS AND REVIEW OF DAIRY LITERATURE

## THE YEASTS<sup>1</sup>

The relation of bacteria to disease probably accounts for the thoroughness with which they have been studied and the number of books which have been written upon bacteria and their activities.

The yeasts are uniformly present in dairy products and play an important part in practically all fermentations, including bread making, and still there is little available literature on yeasts. In fact until recently there has not been a satisfactory text book on this subject.

Appreciating the significance of the recent work of Dr. A. Guillermond, Dr. F. W. Tanner has joined with this author in producing a new text. This text makes available the scattered information concerning the morphology and physiology of yeast, giving particular attention to their cytology, development, nutrition, reproduction and alcoholic fermentation. Additional chapters present the methods of cultivation, of characterization and of identification, followed by chapters on classification and description of known species. The list of described species includes not only the true yeasts but extends into the doubtful yeasts and related fungi.

While it is known that yeasts are always present in fair numbers in dairy products little or nothing is known of the sources from which they enter nor the means best calculated to regulate their activities. Our ignorance in these matters is in considerable part due to the lack of a suitable text for training students to handle and study yeasts. This text will be found of great value in meeting this need.

H. A. HARDING.

<sup>1</sup>By A. Guillermond and F. W. Tanner. John Wiley and Sons, New York, 1920.

## DAIRY NOTES

J. W. HENDRICKSON

*Department of Dairy Husbandry, University of Nebraska, Lincoln, Nebraska*

### COLORADO

The following publications have been completed and sent out from the Dairy Department of the Colorado State College: Bulletin 202 "Testing and Handling of Milk and Cream" by R. McCann, and "Dairy Marketing Survey in Colorado" by J. A. Raitt.

The Station is at present working on the project, "Average Cost of Pasture for Dairy Cows," and when completed they expect to have data of considerable value to the dairymen of Colorado and adjacent states.

Men who were formerly connected with the dairy department of the Colorado State College are now located in the following positions:

R. McCann, National Dairy Council, Chicago, Ill.

E. B. Darrow, Corbett Ice Cream Company, Denver, Colo.

H. J. Haakenson, Fieldman, Boulder Creamery, Denver, Colo.

E. Baker, Colorado Milk Producers Association, Denver, Colo.

D. S. Jordan, Agricultural Professor, Monte Vista, Colo.

Mr. C. N. Shepardson is a member of the Department staff in the position of assistant professor of dairying.

### MASSACHUSETTS

The following changes have taken place in the department during the past year:

Prof. O. A. Jamison resigned his position as associate professor of dairying to take up commercial work as manager of the Attleboro Milk Producers Company, Attleboro, Mass.

Mr. Fred E. Wheeler resigned from his position as instructor of dairying, April 10, 1920 and is now connected with the Milk Producers Dairy Company of Pittsfield, Mass.

Mr. D. L. James gave up the position of extension specialist in dairying, May 22, 1920 in order to become manager of the Fall River Milk Producers Corporation.

Mr. S. E. Van Horn is also connected with the Fall River Milk Producers Corporation since leaving his position as buttermaker in the department.

The following new men have come into the department during the past year: Mr. H. F. Judkins as assistant professor of dairying, November 6, 1919; Mr. T. G. Yaxis, assistant professor of dairying, December 27, 1919; Mr. Glen E. Upton, instructor of dairying, appointed April 28, 1920, and Mr. Adelbert Sheffield took the place of Mr. Van Horn in August 1, 1920. Mr. H. F. Pendleton has recently been placed on the department staff as instructor of dairying.

#### NEW JERSEY

The Dairy Department of the New Jersey Experiment Station is working on a project in coöperation with the United States Dairy Division. This project involves a comparison of line-breeding to out-crossing and of in-breeding to out-crossing. Jerseys are being used for the in-breeding work and Holsteins for the line-breeding in this experiment. A second generation of in-bred animals are now available in the New Jersey herd.

Mr. John Hill, assistant dairy husbandman, resigned July 1, 1920, to go into commercial work. His place is being taken by Mr. S. W. Mead who recently completed his work for Masters Degree at Minnesota.

Mr. Stanley B. Roberts has recently been added to the faculty as assistant dairy specialist.

Mr. W. B. Combs, formerly a member of the New Jersey Dairy Department is now professor of dairying at Pennsylvania Agricultural College.

Mr. L. S. Reford, who formerly occupied a similar position is now at Auburn, N. Y. in commercial work.

#### TEXAS

Mr. J. W. Ridgway resigned as head of the Dairy Husbandry Department June 1, in order to take up work as County Agent of Cooke County, Texas with headquarters at Gainesville.

Mr. R. L. Pou was appointed professor of dairy husbandry, July 1, 1920.

Prof. J. A. Clutter came into the College Station Dairy department during the last year.

The most recent change in the department staff is the addition of Mr. A. L. Darnell, as associate professor of dairying, the appointment being effective September 1, 1920.

#### CHANGES IN UNITED STATES DAIRY DIVISION PERSONNEL

##### *Resignations*

*G. L. Oliver*, dairy husbandman, in charge of dairy extension work in the Middle West, has purchased a farm near Richmond, Va., where he plans to put into practice his knowledge of dairying.

*C. L. Walp*, scientific assistant in dairying, who was employed as office assistant in the dairy farming section, has resigned to go as a partner with Mr. Oliver on his farm near Richmond.

*J. H. McClain*, who has been in charge of southern dairy extension work for twelve years, has resigned to manage his own dairy farm at Campobello, South Carolina.

*O. A. Storricks*, dairy manufacturing specialist, engaged in creamery investigations, has resigned to accept a position as Western Representative for the Gude Brothers Kieffer Company.

*W. M. Clark*, dairy chemist, who has been employed on the chemical investigations in connection with the ripening of Swiss cheese, has resigned to accept a position with the Public Health Service.

*W. G. McGowan*, agent in dairying, engaged in coöperative dairy extension work in Mississippi, has resigned to accept a position in dairy extension work in the State of Virginia.

*W. E. Tomson*, dairy husbandman, engaged in coöperative dairy extension work in the State of Montana, has resigned to accept a position with the University of California.

*Earle Brintnall*, agent in dairying, engaged in coöperative dairy extension work in North Carolina, has resigned to accept a position in dairy extension work in the State of Mississippi.

*H. J. Childress*, agent in dairying, engaged in coöperative dairy extension work in the State of Oklahoma, has resigned to accept the position of county agent in the State of Kentucky.

*W. E. Peterson*, agent in dairying, engaged in coöperative dairy extension work in the State of Kansas, has resigned to accept a position with the Minnesota Holstein-Friesian Breeders' Association.

*Appointments*

*J. A. Conover*, dairy extension worker in this Division 1906-1911, and superintendent of the Naval Academy Dairy, 1911-1920, has been appointed for the organization of bull associations.

*Alan Leighton*, who received his B.S. degree from New Hampshire State College, and has taken graduate work in Cornell University, has been appointed as physical chemist in the Dairy Division laboratories, by transfer from the Bureau of Mines.

*E. L. Westover*, who served as coöperative dairy extension worker in the State of Oregon since May 1, 1917, has resigned to do extension work for the American Guernsey Cattle Club.

*Lee Coe*, coöperative extension cheese worker in North Carolina, died of appendicitis, at Boone, North Carolina, September 15, 1920. Mr. Coe was an expert cheesemaker in New York State previous to his appointment in the Dairy Division, March 1, 1918.



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